JOURNAL OF THE NATIONAL CANCER INSTITUTE

February 1955
Volume 15 • Number 4
A Publication of the
U. S. DEPARTMENT OF
ALTH, EDUCATION, AND WELFARE
Public Health Service
National Institutes of Health

JOURNAL OF THE NATIONAL CANCER INSTITUTE

JOHN R. HELLER, *Editor-in-Chief*
Director, National Cancer Institute

BOARD OF EDITORS

WALTER E. HESTON, *Scientific Editor*

GLENN H. ALGIRE, *Associate Editor*
JONATHAN L. HARTWELL, *Associate Editor*
CARL G. BAKER, *Associate Editor*
ALBERT W. HILBERG, *Associate Editor*
WALTER C. SCHNEIDER, *Associate Editor*

DORIS M. CHANEY, *Managing Editor*
Editorial Assistants
JOAN P. COCHRAN
ISABEL K. NEELY

ADVISERS

G. BURROUGHS MIDER
HOWARD B. ANDERVONT
JESSE P. GREENSTEIN
ROY HERTZ
MURRAY J. SHEAR
MICHAEL B. SHIMKIN
ROBERT R. SMITH
HAROLD L. STEWART
JULIUS WHITE

U. S. DEPARTMENT OF
HEALTH, EDUCATION, AND WELFARE
PUBLIC HEALTH SERVICE
NATIONAL INSTITUTES OF HEALTH
NATIONAL CANCER INSTITUTE
BETHESDA 14, MARYLAND
UNITED STATES OF AMERICA

Medical Library
610.5
U55
N28
v.15
no.1-6

In Memoriam

Egon Karl Ferdinand Lorenz

Former Chief of the Radiation Branch

National Cancer Institute

Egon Lorenz, 1892–1954

This issue of the Journal of the National Cancer Institute is dedicated to the memory of Egon Lorenz, Chief of the Radiation Branch of the National Cancer Institute, who died on February 12, 1954, at the age of 62. At the time of his death, he was widely acknowledged as one of the foremost investigators in the rapidly unfolding field of radiobiology. Some of his many colleagues and friends have contributed papers for this issue as a final tribute to him and to his scientific accomplishments. I am greatly honored to have been invited by the Editor to tell those who did not know him personally something about Egon Lorenz as a scientist and as a man.

Lorenz was above all else an honest and forthright man who disdained pomp, power, and pretense, a man who was usually called "Egon" rather than "Doctor Lorenz" by his associates, young or old. He would undoubtedly have been deeply embarrassed, and would have objected vigorously, at the proposal to honor him here. It seems fitting, therefore, to make this essay simple and informal, in keeping with the personality of Egon himself.

He was born in Breslau, Germany, and received his doctorate in physics at the University of Breslau in 1921. He studied briefly at the University of Zurich and then went to Frankfurt to work with Friedrich Dessauer. Here, in the next few years, he made several significant contributions in radiologic physics, primarily in relation to problems of dosimetry, and designed an early condenser discharge apparatus for high intensity radiography. In 1927, he was awarded a Rockefeller International Research Fellowship and came to this country to continue his research at Harvard with Professor William Duane, then one of the world's greatest radiation physicists.

Lorenz loved Cambridge and decided to stay on after his fellowship tenure was over. The United States Public Health Service had established a small field unit for experimental cancer research at Harvard University Medical School, and in 1930 Dr. J. W. Schereschewsky of the United States Public Health Service, who was in charge of the Laboratory, asked Lorenz to join his staff as its first biophysicist. It was this nucleus of men who, almost 10 years later, moved to Bethesda to carry on their work in the newly created National Cancer Institute. During these early years at Cambridge, Lorenz dropped radiation physics to render general biophysical assistance and consultation to the biologists and chemists of the group, who were occupied with studies on the mechanism of carcinogenesis by the hydrocarbons. He worked out techniques for the spectral analysis of trace residues of these compounds in tumors induced by them, and methods for preparing and administering the hydrocarbons and steroid hormones in colloidal and emulsion form. In the course of these labors, he managed to become a self-taught biologist, and learned to think in biological as well as physical terms.

It was during World War II, however, that his scientific career entered its most intuitive and productive phase, with a return to his original field of interest, radiation and its biological effects. At that time, many international experts, with almost no experimental data available to guide them, had made the educated guess that 0.1 roentgen per 8-hour working day could be tolerated without demon-

820174—55——2

strable injury by those occupationally exposed to ionizing radiation. This was a point about which the infant field of atomic energy could ill afford to guess. Lorenz was asked to participate in the Plutonium Project, and assigned the task of evaluating the biological hazards of radiation at dose levels in the tolerance range. His classic study, conducted under wartime stresses and difficulties, indicated that in several species, and presumably also in man, the accepted tolerance dose was too close to the danger level at which longevity is appreciably reduced and tumor incidence significantly increased. Largely as a result of his findings, the internationally accepted tolerance dose has been revised downward to provide a greater margin of safety.

After the war, the Plutonium Project was reorganized as the Argonne National Laboratory, with which Lorenz retained an affiliation as a consultant. He participated in several radiobiologic investigations with members of its staff, and worked in particular with Dr. Leon Jacobson, of the University of Chicago, in a study of the effects of radiation on the blood-forming tissues. In 1948, Jacobson and his associates made the exciting discovery that mice whose spleens had been exteriorized and lead-shielded during systemic X irradiation failed to die after doses that killed virtually all controls. As this group amplified and extended their work, their findings suggested to Lorenz that the protective activity of the mouse spleen might be related to its important role as an extramedullary hematopoietic organ. Accordingly, he injected mouse bone marrow into irradiated mice of the same strain, without spleen shielding, and found that it prevented postirradiation death in exactly the same way. Subsequently, he and his colleagues found that marrow and bone from other strains, and even from other species, also conferred protection, though of lesser degree. He was working actively on the biological characterization of the active factor in marrow at the time of his death.

Although they were perhaps overshadowed by his work on bone marrow, a number of other highly important lines of radiobiologic research were developed by Lorenz during the postwar years. He extended his earlier work on chronic radiation injury; observed and described the development of cataracts in mice exposed at birth to relatively small X-ray doses; studied the distribution, excretion, and dosimetry of injected radium and radon in mice, and compared the biological effectiveness of their α-rays with that of 200 kv. X rays; investigated the mechanism of carcinogenesis by radiation, with particular emphasis on lymphatic leukemia and lymphosarcoma, and ovarian, mammary, and pulmonary tumors; and explored afresh the field of experimental radiation therapy of tumors. In the last-named study, he discovered that small doses of whole-body irradiation can synergize with local irradiation to enhance regression, and even to effect permanent cure, of transplanted lymphosarcomas in mice.

He led an active and productive section, in which several quite unrelated approaches to the cancer problem have been under study by independent senior investigators, whom he very carefully left alone. He avoided domination of his group, and encouraged and developed initiative and independence in its junior members. In no sense an empire builder interested in personal power or kudos, he fought vigorously for improved facilities, more advanced equipment, and an augmented

budget for his section whenever he felt that its scientific productivity might otherwise be curtailed. At scientific meetings at home and abroad, he was an impressive and much respected representative of the best ideals and traditions of the National Cancer Institute.

Let us try now to form some picture of Egon Lorenz as a man. Of moderate height, his slender rugged frame gave one the impression of a relatively tall, athletic man. He was an emotionally intense man who habitually maintained his emotions under tight control. In the company of friends, he had a ready and hearty laugh and a gay, almost boyish charm. Egon had tremendous drive and vitality, and despite a heart attack in 1945 that left him subject to disabling anginal pain, could regularly be found in the laboratory or at his desk on weekends and after hours. The heart attack was particularly tragic for him because it brought to an end the skiing, mountain climbing, and outdoor life he had so actively enjoyed till then. He refused, however, to give up another early enthusiasm, dancing; he was gifted with an excellent sense of rhythm, was particularly adept in the tango, and readily learned intricate native dances on his foreign trips. He loved the rugged beauties of nature, especially the snow-capped peaks of the Alps and the Andes, and the crash of ocean waves on a rocky seashore. Egon and Marjorie, his young wife with whom he enjoyed a few brief years of marital happiness before his death, particularly delighted in strolling along the relatively deserted beaches of Nantucket in early summer. Because of his cardiac illness and his aversion for large social gatherings, they led a rather secluded and simple life during these years. Yet, paradoxically, Egon Lorenz was a man who lived his life to the fullest and knew how to savor and enjoy the process of living. His close friends were few because he regarded friendship as a precious element of life; to them he was intensely devoted and sympathetic. Those who were fortunate enough to know him intimately must feel particularly keenly the great loss which his death represents for all scientists in the fields to which he contributed so much.

Henry S. Kaplan

Bibliography of Egon Lorenz

1921–1925

Exakte Registriermethode für Messungen im Ultraroten.
Thesis, Universität Breslau, 1921.

Measurements of absorption coefficient of water and aluminum for hard x-rays. (With B. Rajewsky.)
Am. J. Roentgenol. 10: 890–896, 1923.

In German.
Strahlentherapie 16: 475–486, 1924.

Die Rolle der Streuung für die Strahlenwirkung unter Berücksichtigung des Compton-Effektes. (With B. Rajewsky.)
Strahlentherapie 18: 473–480, 1924.

Über einige Wirkungen von Strahlen. V. Die Bedeutung des Compton-Effektes für die Wirkung der Röntgenstrahlen. (With B. Rajewsky.)
Ztschr. Phys. 27: 32–36, 1924.

Zur Frage der Dosierung von Röntgenstrahlen. (With B. Rajewsky.)
Ztschr. ges. physik. Therapie 29: 123–146, 1924.

Zur Frage der Qualifizierung von Röntgenstrahlen. (With B. Rajewsky.)
Strahlentherapie 19: 349–357, 1925.

Das Problem der Intensitätsverteilung von Röntgenstrahlen im durchstrahlten Medium. (With B. Rajewsky.)
Strahlentherapie 20: 581–631, 1925.

1926–1930

Über einen Universal-Gleichspannungsapparat für Roentgen-Diagnostik und Therapie. (With M. Brenzinger and F. Dessauer.)
Fortschr. Geb. Röntgenstrahlen 34: 152–158, 1926.

Über ein Instrumentarium zur Erzeugung von hochgespanntem Gleichstrom. (With M. Brenzinger and F. Dessauer.)
Ztschr. tech. Physik 7: 84–90, 1926.

Das Problem der Intensitätsverteilung von Röntgenstrahlen im durchstrahlten Medium (With B. Rajewsky.)
Strahlentherapie 24: 175–191, 1927.

The standard ionization chamber for roentgen ray dosage measurements. (With W. Duane.)
Am. J. Roentgenol. 19: 461–469, 1928.

In German
Fortschr. Geb. Röntgenstrahlen 37: 691–696, 1928.

The spectrum of x-rays from the back of a tungsten target.
Proc. Nat. Acad. Sc. 14: 582–588, 1928.

Über die Intensität der Röntgenspektrallinien, insbesondere der K=Serie des Aluminums, in Abhängigkeit von der Röhrenspannung.
Ztschr. Physik 51: 71–94, 1928.

Zur Frage der Streustrahlung der Luft. (With B. Rajewsky.)
Fortschr. Geb. Röntgenstrahlen 38: 1124, 1928.

The development of dosimetry. An historical sketch.
Radiology 14: 340–345, 1930.

1933–1937

Investigation of mitogenetic radiation by means of a photoelectric counter tube.
Pub. Health Rep. 48: 1311–1318, 1933.

Search for mitogenetic radiation by means of the photoelectric method.
J. Gen. Physiol. 17: 843–862, 1934.

Studies in carcinogenesis. II. The detection of dibenzanthracene in mouse tumors induced by this hydrocarbon. (With M. J. Shear.)
Am. J. Cancer 26: 333–343, 1936.

Note on the preparation of dispersions of carcinogenic hydrocarbons in serum. (With H. B. Andervont.)
Am. J. Cancer 26: 783–786, 1936.

Experimental siderosis. III. Spectroscopic studies of iron-containing pigment. (With V. Menkin.)
Arch. Path. 22: 82–85, 1936.

Dibenzanthracene tumors in mice. The production of subcutaneous, pulmonary, and liver tumors by serum dispersions and lard solutions. (With H. B. Andervont.)
Pub. Health Rep. 52: 637–647, 1937.

Dibenzanthracene tumors in mice. The production of subcutaneous and pulmonary tumors by 1:2:5:6-dibenzanthracene adsorbed on charcoal. (With H. B. Andervont.)
Pub. Health Rep. 52: 1931–1940, 1937.

1939–1941

"Double beam" roentgen therapy. (Simultaneous irradiation through two portals at opposite sides.) (With H. F. Friedman.)
Am. J. Roentgenol. 41: 637–641, 1939.

Studies in carcinogenesis. VI. Hydrocarbon-cholesterol pellets in Albino mice. (With M. J. Shear.)
Am. J. Cancer 36: 201–210, 1939.

Incidence of induced pulmonary tumors in susceptible mice raised in dust-free air. (With H. B. Andervont.)
Arch. Path. 29: 484–493, 1940.

Intestinal carcinoma and other lesions in mice following oral administration of 1, 2, 5, 6-dibenzanthracene and 20-methylcholanthrene. (With H. L. Stewart.)
J. Nat. Cancer Inst. 1: 17–40, 1940.

Squamous cell carcinoma and other lesions of the forestomach in mice, following oral administration of 20-methylcholanthrene and 1, 2, 5, 6–dibenzanthracene. (With H. L. Stewart.)
J. Nat. Cancer Inst. 1: 273–276, 1940.

Preparation of dispersions of carcinogenic hydrocarbons and hormones with the aid of dioctyl ester of sodium sulfosuccinate (Aerosol O. T.). (With M. B. Shimkin and H. L. Stewart.)
J. Nat. Cancer Inst. 1: 355–360, 1940.

Induction of adenocarcinoma of the pyloric stomach in mice by methylcholanthrene. (With H. L. Stewart.)
J. Nat. Cancer Inst. 2: 193–196, 1941.

1942–1944

Disappearance of intravenously injected methylcholanthrene in mice of different susceptibility to pulmonary tumors. (With M. B. Shimkin.)
J. Nat. Cancer Inst. 2: 491–498, 1942.

Factors influencing the induction of pulmonary tumors in strain A mice by carcinogenic hydrocarbons. (With M. B. Shimkin.)
J. Nat. Cancer Inst. 2: 499–510, 1942.

Adenocarcinoma of the pyloric stomach and other gastric neoplasms in mice induced with carcinogenic hydrocarbons. (With H. L. Stewart.)
J. Nat. Cancer Inst. 3: 175–189, 1942.

Hydrogen discharge tube with metal capillary. (With P. D. Kueck.)
J. Optical Soc. Am. 33: 663–665, 1943.

Radioactivity and lung cancer; a critical review of lung cancer in the miners of Schneeberg and Joachimsthal.
J. Nat. Cancer Inst. 5: 1–15, 1944.

Absorption of subcutaneously implanted hormone pellets. (With M. B. Shimkin, R. Wyman and S. G. Norton.)
Endocrinology 35: 283–295, 1944.

1946–1947

Increase in incidence of lung tumors in strain A mice following long-continued irradiation with gamma rays. (With W. E. Heston, M. K. Deringer and A. B. Eschenbrenner.)
J. Nat. Cancer Inst. 6: 349–353, 1946.

Counting rate and frequency meter. (With J. Weikel and S. G. Norton.)
Rev. Scient. Instruments 17: 276–279, 1946.

Tumors of the alimentary tract induced in mice by feeding olive-oil emulsions containing carcinogenic hydrocarbons. (With H. L. Stewart.)
J. Nat. Cancer Inst. 7: 227–238, 1947.

Histopathology of induced precancerous lesions of the small intestine of mice. (With H. L. Stewart.)
J. Nat. Cancer Inst. 7: 239–268, 1947.

Biological studies in the tolerance range. (With W. E. Heston, A. B. Eschenbrenner and M. K. Deringer.)
Radiology 49: 274–285, 1947.

1948–1949

Excretion of radium from the mouse. (With J. Weikel.)
Radiology 51: 865–870, 1948.

Quantitative histologic analysis of the effect of chronic whole-body irradiation with gamma rays on the spermatogenic elements and the interstitial tissue of the testes of mice. (With A. B. Eschenbrenner and E. Miller.)
J. Nat. Cancer Inst. 9: 133–147, 1948.

Tumors of alimentary tract in mice fed carcinogenic hydrocarbons in mineral-oil emulsions. (With H. L. Stewart.)
J. Nat. Cancer Inst. 9: 173–180, 1948.

Lack of effect of adrenalectomy on the lymphopenic response of C57 black mice to total-body irradiation at a low dosage level. (With S. N. Marder and H. S. Kaplan.)
Quarterly Report, Biological and Medical Divisions, Argonne National Laboratory, August to November, 1948. (ANL-4227), pp. 21–27.

The hematological effects of ionizing radiations. (With L. O. Jacobson and E. K. Marks.)
Radiology 52: 371–395, 1949.

Retention of radon by the mouse—1. Experimental determination of biodecay and energy absorbed. (With J. W. Hollcroft.)
Nucleonics 5: 63–71, 1949.

Morbid anatomy, histopathology, and histopathogenesis of forestomach carcinoma in mice fed carcinogenic hydrocarbons in oil emulsions. (With H. L. Stewart.)
J. Nat. Cancer Inst. 10: 147–166, 1949.

Preparation of emulsions and suspensions containing carcinogenic hydrocarbons.
J. Nat. Cancer Inst. 10: 355–358, 1949.

Some biologic effects of ionizing radiations on laboratory animals.
Actas del Tercer Congreso Interamericano de Radiologia, Chile, 1949, pp. 380–387.

1950

Some biologic effects of long continued irradiation.
Am. J. Roentgenol. 63: 176–185, 1950.

Ocular lesions induced by acute exposure of the whole body of newborn mice to roentgen radiation. (With T. B. Dunn.)
Arch. Ophth. 43: 742–749, 1950.

Studies of the effects *in vitro* of roentgen radiation on the biological activity of the agent of chicken tumor I (Rous sarcoma). (With W. R. Bryan and J. B. Moloney.)
J. Nat. Cancer Inst. 10: 1215–1237, 1950.

Effects of ionizing radiations on a transplanted lymphosarcoma. (With J. Hollcroft and H. Hunstiger.)
J. Nat. Cancer Inst. 11: 1–16, 1950.

1951

Hematological effects of ionizing radiations. (With L. O. Jacobson and E. K. Marks.) In *Industrial Medicine on the Plutonium Project, Vol. 20, National Nuclear Energy Series, Manhattan Project Technical Section, Division IV—Plutonium Project Record (R. S. Stone, ed.). New York, McGraw-Hill Book Co., Inc., 1951, Chapt. 5, pp. 140–196.*

Recovery pattern of the blood picture in guinea pigs following a limited total body exposure to chronic gamma radiation.
J. Chimie Physique 48: 264–274, 1951.

Mammary-tumor incidence in female C3Hb mice following long-continued gamma irradiation. (With A. B. Eschenbrenner, W. E. Heston and D. Uphoff.)
J. Nat. Cancer Inst. 11: 947–965, 1951.

An application of mass spectrometer analysis to the study of respiratory metabolism. (With A. W. Pratt, B. E. Burr and M. Eden.)
Rev. Scient. Instruments 22: 694–696, 1951.

Modification of irradiation injury in mice and guinea pigs by bone marrow injections. (With D. Uphoff, T. R. Reid and E. Shelton.)
J. Nat. Cancer Inst. 12: 197–201, 1951.

The 30-day LD-50 of two radiations of different ion density. (With J. W. Hollcroft.)
J. Nat. Cancer Inst. 12: 533–544, 1951.

1952

Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. (With C. Congdon and D. Uphoff.)
Radiology 58: 863–877, 1952.

Factors modifying the effect of X-irradiation on regression of a transplanted lymphosarcoma. (With J. W. Hollcroft and M. Matthews.)
J. Nat. Cancer Inst. 12: 751–763, 1952.

Modification of acute irradiation injury in mice and guinea pigs by injection of bone marrow: A histopathologic study. (With C. C. Congdon and D. Uphoff.)
J. Nat. Cancer Inst. 13: 73–107, 1952.

Tumors of the glandular stomach induced in rats by intramural injection of 20-methylcholanthrene. (With W. V. Hare, H. L. Stewart and J. G. Bennett.)
J. Nat. Cancer Inst. 12:1019–1055, 1952.

Modification of acute and chronic irradiation injury in experimental animals by injection of bone marrow and tissues originating from hematopoietic organs. (With C. C. Congdon.)
Proc. Fourth International Congress of the International Society of Hematology, Argentina, 1952, pp. 192–211.

1953

Factors influencing the irradiation treatment of experimental lymphoid tumors. (With J. Hollcroft, C. C. Congdon and L. O. Jacobson.)
Revista Mexicana de Radiologia 7: 115–124, 1953.

Protecting action of citrate on the agent of chicken tumor I (Rous sarcoma virus) during roentgen radiation *in vitro*. (With W. R. Bryan and D. Calnan.)
J. Nat. Cancer Inst. 13: 1447–1461, 1953.

Occurrence of pulmonary tumors in strain A mice following total-body X-radiation and injection of nitrogen mustard. (With W. E. Heston and M. K. Deringer.)
Cancer Res. 13: 573–577, 1953.

Prevention of irradiation-induced lymphoid tumors in C57BL mice by spleen protection. (With C. C. Congdon and D. Uphoff.)
J. Nat. Cancer Inst. 14: 291–301, 1953.

1954

Humoral factor in irradiation protection: Modification of lethal irradiation injury in mice by injection of rat bone marrow. (With C. C. Congdon.)
Am. J. Physiol. 176: 297–300, 1954.

Modification of lethal irradiation injury in mice by injection of homologous or heterologous bone. (With C. C. Congdon.)
J. Nat. Cancer Inst. 14: 955–965, 1954.

Leukemia in guinea-pigs. (With C. C. Congdon.)
Am. J. Path. 30: 337–359, 1954.

Biologic and physical aspects of the action of two radiations of different specific ionization. (With J. W. Hollcroft.)
Am. J. Roentgenol. 72: 124–132, 1954.

Radioactivity: Biologic effects of ionizing radiations. (With C. C. Congdon.)
Ann. Rev. Med. 5: 323–338, 1954.

Carcinogenesis by ionizing radiations. (With J. Furth.)
In *Radiation Biology, Vol. I: High Energy Radiation (A. Hollaender, ed.). New York, McGraw-Hill Book Company, Inc., 1954, Chapt. 18, pp. 1145–1201.*

The role of bone marrow and spleen in induced and spontaneous lymphatic leukemia. (With L. W. Law and C. C. Congdon.)
In *CIBA Foundation Symposium on Leukemia Research (G. E. W. Wolstenholme and M. P. Cameron, eds.) London, J. and A. Churchill, Ltd., 1954, pp. 189–195.*

Some aspects of the role of hematopoietic tissues in the pathogenesis and treatment of experimental leukemia. (With C. C. Congdon.)
Proceedings of the Vth International Congress on Hematology. Paris, 1954.

Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. I. Preliminary experiments. (With W. E. Heston.)
In *Biological Effects of External X and Gamma Radiation, Vol. 22B. Part I. National Nuclear Energy Series. Manhattan Project Technical Section. Division IV—Plutonium Project Record (R. E. Zirkle, ed.). New York, McGraw-Hill Book Co., Inc., 1954, Chapt. 1, pp. 1–11.*

Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. II. Physical Arrangement.
Ibid, *Chapt. 2, pp. 12–23.*

Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. III. Effects on life span, weight, blood picture, and carcinogenesis and the role of the intensity of radiation. (With L. O. Jacobson, W. E. Heston, M. Shimkin, A. B. Eschenbrenner, M. K. Deringer, J. Doniger, and R. Schweisthal.)
Ibid, *Chapt. 3, pp. 24–148.*

Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. IV. Actions on the breeding behavior of mice. (With M. K. Deringer and W. E. Heston.)
Ibid, *Chapt. 4, pp. 149–168.*

Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. VI. Conclusions and applicability of results to the problem of human protection.
Ibid, *Chapt. 6, pp. 226–242.*

JOURNAL OF THE NATIONAL CANCER INSTITUTE

NATIONAL INSTITUTES OF HEALTH • Public Health Service

Volume 15
Number 4

February 1955

The Histopathology of Bacterial Infection in Irradiated Mice [1]

CHARLES C. CONGDON, FLETCHER P. WILLIAMS, JR., ROBERT T. HABERMAN, *and* EGON LORENZ,[2,3]
National Cancer Institute and National Institute of Arthritis and Metabolic Diseases,[4] *Bethesda, Md.*

The tissue changes associated with the bacterial infection occurring in irradiated mice are of special interest since the element of purulent exudation is lacking in the inflammatory lesions. The bacteriologic findings have indicated that infection is a major factor in causing death of mice after total-body irradiation over a wide dose range (*1–3*). Invasion from the intestinal tract is considered the portal of entry since the types of bacteria present in the blood and tissues are normally present in the intestine (*1–3*).

The disease is probably independent of the type of ionizing radiation to which animals are exposed, since the bacterial infection occurs after exposure to X rays, *gamma* rays, or neutrons (*2*, *4*). Injection of normal bone marrow into irradiated mice prevented the bacterial infection (*5*, *6*). The present work was undertaken to determine the histopathology of the infection and to demonstrate morphologic lesions in the intestine from which bacteria gain entrance into the blood stream.

Experimental Groups and Procedures

Two groups of mice were used for histologic examination. The irradiation technique was the same for both.[5]

Group 1.—Twenty-nine male and 28 female C3Hf mice, 5 to 5½ months of age, were given 900 r total-body irradiation. All animals were irradiated on the same day. Five mice were killed and autopsied each day after irradiation, except days 5 and 7, through the 10th day. On days 5 and 7, three animals were killed and autopsied each day; and, in addition, eight mice found dead on the 5th day and three mice found dead

[1] Received for publication July 23, 1954.

[2] Deceased February 12, 1954.

[3] The authors are indebted to Bruce L. Weed, Beverly Jensen, Eliza Miller, and Henry Meyer for technical assistance.

The C3Hf mice used in these experiments were provided through the kindness of Dr. Walter E. Heston and Dr. Margaret D. Barrett.

[4] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

[5] The conditions were two tubes opposite each other to obtain a uniform tissue dose; their foci were 54 cm. from the center of the mice. 0.25 mm. Cu + 1.06 mm. Al filtration of the X-ray beams operating at 186 KVP and 20 Ma. was used. The dosage rate was 93 r (air) per minute.

on the 7th day were autopsied. Five normal female C3Hf mice were killed and autopsied during the course of this experiment.

The tissues taken at autopsy included all major organs and tissues in the cranial, thoracic, and abdominal cavities. The neck organs were rarely examined. Tissues were fixed in Spuler's formalin variant of Zenker's solution and sections prepared from them were stained with hematoxylin and eosin. A duplicate set of sections was stained with a gram stain (*7*).

Group 2.—The remaining histologic material came from about 700 autopsies of mice from several inbred strains and two homogenous F_1 hybrids [C3Hf, A, C57BL, C57L, BALB/cAn, LAF_1, (C57BL × DBA)F_1] that had been exposed to 800 r or 900 r total-body irradiation. Some of these mice served as irradiation controls; others had received injections of bone marrow or other tissues, after the lethal dose of total-body irradiation. Still others had received chemical agents thought to stimulate hematopoiesis. Most of these experiments in which these mice were used have been reported elsewhere (*5*, *8*, *9*). One hundred nonirradiated mice were also killed and autopsied in the course of different experiments. These were examined for bacterial infections.

TABLE 1.—*Frequency of bacterial lesions as observed in*

Day†	3					4					6										
Tissues Sex	F	M	M	M	F	M	M	M	M	M	F	M	M	M‡	M‡	M‡	M‡	M‡	F‡	F‡	F‡
Small intestine	.	.	x	x	x	x	x	x	x	.	.	.	x	.	x	.	.	.	.	x	.
Peyer's patch	.	.	.	.	.	.	.	.	x	.	.	.	x	.	.	.	.	.	.	x	.
Large intestine	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Spleen	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	x	.	.	x	x	x
Liver	.	.	.	.	.	.	.	.	.	x	.	.	.	.	x	x	.	x	x	x	x
Kidney	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	x	.	.	.	.
Adrenal	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Heart	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.
Lung	.	.	.	.	.	.	.	.	.	x	.	.	.	x	.	x	x	.	x	x	.
Brain	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	.	.	.	.	.	.
Bone marrow	.	.	.	.	.	.	.	.	.	x	.	.	.	.	.	x	.	.	x	x	x
Lymph node	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.	x	x	x
Striated muscle	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Pancreas	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.
Urinary bladder	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	.	x	.	.	.	.

*x = One or more bacterial lesions observed in the routine hematoxylin and eosin, and Brown and Brenn, stain sections made of the tissues.

The autopsy material was reviewed from the standpoint of bacterial infection. Much of the material came from animals found dead in their cages; some were sacrificed when found dying and some represented serial sacrifice in bone-marrow-injected animals. The great majority of the tissues were fixed in Spuler's formalin—variant of Zenker's solution. Occasionally 10 percent formalin was used. Hematoxylin and eosin sections were made from the tissues. A few selected animals were used for gram-staining of tissues.

Results

Group 1.—No gross or histologic evidence of bacterial infection was observed in the five normal mice killed and autopsied. The gross findings directly associated with the bacterial lesions in the irradiated animals were limited to the observation of gray-white focal areas of necrosis in the livers of some animals. Mottled red areas of discoloration in the lungs were usually associated with the presence of bacterial infection in this organ. Hemorrhagic areas in spleen, brain, and testis were sometimes associated with microscopic evidence of bacterial infection, but other times this was not the case.

Table 1 shows the distribution of bacteria in tissues and organs as determined by study of histologic sections.

*histologic sections of serially autopsied, irradiated mice**

6					7						8					9					10				
M	M	F	F	F	M	M	M	F‡	F‡	F‡	F	F	F	F	M	M	M	M	M	F	F	F	F	F	M
...	..	..	..	..	...	x	...	...	...	...	..	..	..	..	x	...	...	x	x	..	..	x	..	..	x
...	..	..	..	..	...	x	...	...	...	...	..	..	..	..	x	...	...	...	...	..	..	..	..	..	..
...	..	..	..	..	...	...	...	...	...	...	..	..	..	..	...	...	...	...	x	..	..	..	..	..	..
...	..	x	x	x	...	...	x	x	...	x	..	..	..	..	...	x	...	...	...	..	..	..	..	..	x
...	..	x	..	..	...	...	x	x	x	x	..	..	x	x	...	x	x	...	...	x	x	x	..	..	x
...	..	..	x	..	...	...	...	x	x	...	..	..	..	..	...	...	x	x	x	..	..	..	..	..	..
...	..	..	..	..	...	...	...	x	...	x	..	..	..	..	...	...	...	...	...	..	..	..	..	..	..
...	..	..	..	..	...	...	...	...	...	...	..	..	..	..	...	...	...	x	x	..	..	..	..	..	..
...	..	x	x	..	...	...	...	x	...	x	..	..	..	x	...	x	x	x	x	..	x	x	x	x	x
...	..	..	..	..	...	...	...	...	...	...	..	..	..	..	...	...	...	x	x	..	..	..	..	..	..
...	..	x	x	..	...	...	x	x	x	x	..	..	..	..	...	x	...	...	...	..	..	..	..	..	..
...	..	..	..	..	...	...	...	...	...	x	..	..	..	..	x	...	x	x	...	..	..	..	..	..	..
...	..	..	x	..	...	...	...	...	...	...	..	..	..	..	...	x	...	...	...	..	..	..	..	..	..
...	..	..	..	..	...	...	...	...	...	...	..	..	..	..	...	...	...	x	...	..	..	..	..	..	..
...	..	..	..	..	...	...	...	...	...	...	..	..	..	..	...	...	...	...	...	..	..	..	..	..	..

†No bacteria observed on days 1 and 2.
‡Found dead.

Histologically, bacteria were not observed in the tissues of animals sacrificed before the 3rd postirradiation day. In these early animals, however, the destruction of hematopoietic tissue and intestinal epithelium at 24 and 48 hours after irradiation was already well advanced. The first evidence of bacteria in tissues in this series was observed in the intestinal epithelium on the 3rd and 4th days following irradiation. In two of the mice on the 3rd day, isolated bacteria were seen in the altered mucosal cells of the small intestine. In one mouse, a small clump of bacteria was found beneath the mucosal surface in the tissue fluid. On the 4th day, one mouse showed many isolated cocci scattered through the mucosal epithelial cells (figs. 1 and 2). Two other mice showed mucosal lesions and one animal had a large necrotizing bacterial lesion (figs. 3 to 8). On subsequent days, the occasional lesions observed in the small intestines were of two types. In several mice, an ulcer was observed at the site of a Peyer's patch with large masses of bacteria in the ulcer base (fig. 9). In four other mice, there were bacterial masses in blood vessels of the intestinal mucosa. This latter type of bacterial lesion was essentially identical with that present in all other tissues in which bacteria were found.

In the organs and tissues other than small intestine listed in table 1, the earliest evidence of bacterial infection consisted of small bacterial masses in capillaries or sinusoids. Occlusion of the blood vessel occurred as the bacterial mass enlarged and the vessel wall underwent necrosis at one or more points. At the same time, either through direct invasion by bacteria or through diffusion of toxins, the adjacent tissues usually showed degenerative changes and necrosis of cells. Occasionally a bacterial mass produced no apparent injury to the adjacent vessel wall or tissues. Exudation of leukocytes was never observed since these cells were nearly entirely absent from the blood stream. In the lung, a serous and hemorrhagic exudate appeared in the aveolar spaces. A fibrin network was sometimes observed throughout a bacterial lesion. The finding of bacterial colonies in this group and in Group 2 in areas of hemorrhage in the brain, testis, and in various submucosal tissues led to the suggestion that hemorrhage per rhexin occurred from bacterial necrosis of vessel walls. Direct proof of this, however, was never observed.

The frequency with which different organs showed bacterial lesions (table 1) demonstrates that lungs and liver were more frequently involved than other organs and tissues. Spleen, kidney, bone marrow, lymph nodes, and small intestine often showed involvement. Invasion of other tissues was relatively rare in this experimental group.

The character of the bacterial lesions at different days after irradiation was relatively constant, although some of the most extensive lesions were observed in animals sacrificed on days 8 through 10. The distribution of the lesions after day 4 remained relatively constant. In the lungs, the earliest lesions consisted of tiny collections of bacteria in alveolar capillaries (fig. 10). Growth of bacteria produced either masses extending along the capillary lumen surrounding alveolar spaces or nodular masses

of bacteria overgrowing alveolar walls and spaces in all directions. Necrosis of tissue was usually present (fig. 11). The most extensive involvement of the lungs consisted of a patchy growth of bacteria in capillaries throughout an entire lobe of the lung (fig. 12). In these cases, the alveolar spaces were filled with serous and hemorrhagic exudate. Reparative processes were not observed.

In the liver, the smallest bacterial foci were observed in the sinusoids. Enlargement of the lesion occurred either as a nodular mass of bacteria which destroyed and overgrew a small area of the liver or as growth of bacteria along sinusoids and into central veins, producing a lesion in which bacterial masses outlined the vascular channels of the area. Simple necrosis of liver-cord cells in the involved areas was always present (fig. 13).

The bone marrow, lymph nodes and spleen collectively formed a large percentage of the sites of bacterial growth and bacterial necrosis.

In the bone marrow, the smallest areas of bacterial growth and necrosis originated from sinusoids. Diffuse areas of bacterial growth and necrosis developed from these. In some cases, the bacterial growth tended to follow the pattern of the sinusoids. The structure of the trabecular bone was not altered by the bacterial infection.

The earliest lesions associated with bacteria in the spleen were in the sinusoids of the red pulp. More extensive lesions showed diffuse areas of necrosis containing colonies and isolated bacteria (fig. 14). Intense congestion of and hemorrhage into the spleen of an irradiated animal usually was associated with the presence of bacterial necrosis.

The lymph nodes examined were usually peripheral ones, including those from the inguinal, axillary, and cervical regions. Visceral lymph nodes such as mediastinal, pancreatic, and mesenteric were not regularly examined in this particular series. The earliest lesions observed were in the peripheral sinus of a lymph node or in small vessels. The former locus was most common. More advanced lesions showed patchy areas of necrosis resulting from the bacterial growth and spread; and occasionally the entire node was the site of bacterial necrosis.

The visceral lymph nodes examined, including the mesenteric, in this particular group showed a few bacterial lesions.

In the kidneys, bacterial lesions were usually confined to the cortex and showed localization in glomerular capillaries as well as in those between the convoluted tubules. Growth of bacteria along the capillary occurred, as well as destruction of the capillary wall and adjacent tissue. In one case, bacterial masses plugged small arteries within the kidney, and in another, a bacterial colony was lodged against the wall of the vein.

The pattern of the bacterial lesions described for the above tissues applies to the remainder of the organs and tissues listed in table 1, with the notable rare exception of bacterial colonies in an area of hemorrhage in the brains of two animals.

Only once were bacterial colonies found in large vessels in this series of autopsies. These were observed in the pulmonary arteries and arteries

of the kidney in an animal killed and autopsied on the sixth day after irradiation.

The gram-staining reactions of the bacteria in the tissues showed some lesions to be entirely gram-positive and some entirely gram-negative. Not infrequently, the staining reactions were inadequate to show the true gram character of the bacteria. In any given animal, lesions in different organs showed the same gram reaction.

In the tissues, the bacteria observed consisted of cocci or short rods. Specific lesions usually appeared to contain bacteria of only one morphologic type.

A few of the animals listed in table 1 showed no histologic evidence of bacterial infection.

Group 2.—In addition to the series of approximately 700 autopsies of irradiated mice, there were 100 animals not irradiated. None of these showed histologic evidence of bacterial infection. In the irradiated mice that died of the irradiation syndrome, bacterial infection was a very common histologic finding. The bacterial infection showed essentially the same features in the different inbred strains and hybrid mice examined. A finding in the LAF_1 hybrid, which was unusual in other types, was the extreme edema of the face, head, and neck that these particular mice commonly developed prior to death. Histologically, these swollen subcutaneous areas were made up of edema fluid and usually showed bacterial colonies in the fluid and tissues. In the neck region, the salivary glands and lymph nodes were usually also involved in the inflammatory edema.

In one group of 15 LAF_1 mice that had received 900 r and no other treatment, sections were made near the time of death from the mesenteric lymph node to determine if this structure always showed bacterial lesions. Approximately half of the mice showed the presence of a bacterial lesion.

Extensive destructive lesions of the intestinal wall were observed in only two mice. Diffuse bacterial growth was present in both of these lesions. The intestinal ulcer usually at the site of a Peyer's patch seen in several animals in Group 1 was occasionally seen in this larger series of mice in Group 2. A few animals in this series (Group 2) showed massive pleural or epicardial growth of bacteria. Some of the most extensive bacterial masses observed occurred in irradiated animals that had received injection of foreign materials.

Post-mortem changes frequently had to be differentiated from antemortem bacterial growth. This was usually possible when necrosis or slight hemorrhage was observed in the same lesion.

The relationship of hemorrhage to bacterial infection was considered in this material. Large hemorrhages into testis, submucosa of the urinary bladder, cerebral meninges, sites of trauma and sometimes lungs are occasionally seen in mice dying of the irradiation syndrome. Bacteria and bacterial colonies are sometimes seen in the area of hemorrhage. It is not clear whether the bacteria lodging in a small blood vessel causes its rupture and the subsequent hemorrhage or whether the hemorrhagic tissue provides a site for bacterial growth.

In Group 2 thirteen animals showed bacteria in phagocytes of the spleen, bone marrow, and sometimes lymph nodes and liver. The bacteria were identified by their morphology and gram-staining reaction. All of these animals died of the irradiation syndrome with extensive bacterial infection of the type commonly seen with lesions originating in blood vessels.

Discussion

The lesions observed histologically in the intestinal epithelium include the presence of bacteria in injured epithelial cells and necrotizing bacterial ulcers. The presence of bacteria in the epithelial cells was observed earlier by Chrom (*1*) and Mottram (*10*). The intestinal ulcers have been observed previously by Lawrence and Tennant (*2*).

Since all evidence points to the intestine as the portal of entry, these lesions observed in the intestine are presumably one mechanism by which the entrance of bacteria actually takes place. There is some difficulty, however, in explaining the findings at different exposure levels with regard to the onset of bacteremia. At 900 r lesions in the intestinal epithelium (table 1) capable of causing the bacteremia were found from the third postirradiation day on. They ranged from tiny microscopic necrotic areas to large bacterial ulcers. In the recent work by Hammond *et al.* (*11*), where a dose of 550 r was used, the bacteremia showed an onset at approximately the 7th postirradiation day. Presumably in their mice the lesions in the intestinal mucosa were present in some form for several days before the bacteremia, if the findings reported in this paper apply to their work. This assumes that the intestinal bacterial lesions reported here are a consequence of focal, incomplete regeneration of the mucosa following the irradiation damage.

In another paper Hammond *et al.* (*12*) studied the recovery of *Pseudomonas* organisms at autopsy when the mice, given 550 r, were inoculated intragastrically with a *Pseudomonas* culture at different time intervals postirradiation. They found the least recovery of *Pseudomonas* at autopsy, when the organisms were administered immediately after the X-ray exposure, and the greatest recovery when administered 5 and 11 days after the irradiation exposure. Since the intestinal damage is generally considered healed by these later time intervals, the authors felt that the data suggested penetration of an intact mucosa by the administered bacteria.

It seems entirely possible that the administered *Pseudomonas* organisms may cause intestinal lesions, even though they do not kill nonirradiated mice. This does not explain their findings at the earliest time interval, however. Of further interest in this regard is the work of Schweinburg, *et al.* (*13*), who demonstrated in dogs that living or dead *Escherichia coli* tagged with radioactive iodine could pass directly through the intestinal wall into the peritoneal cavity when the peritoneum was irritated by a lavage procedure.

Since all the data dealing with the mode of entry of bacteria into the irradiated animal are not explained by the specific lesions demonstrated

here, it is necessary to reserve complete judgment on the matter and consider that passage of bacteria through intact epithelium, as well as specific lesions, may be involved in the pathogenesis of the bacteremia.

The inflammatory edema of the head and neck in irradiated LAF_1 mice observed in the current work suggests the mouth as another portal of entry for this particular bacterial lesion.

The pathogenesis of the bacterial infection probably depends upon the multiplication of the bacteria gaining direct entrance into the blood stream or indirectly through the lymphatic apparatus of the gut. The inability of the blood stream to clear the organisms results in their settling out in capillaries, sinusoids, and lymph spaces throughout the body. Rarely, the irradiated mice show ability to phagocytose bacteria as was observed in a few animals of Group 2. The growth of bacteria in small vascular channels and the spread of the bacteria along the channel or through the wall into adjacent tissues constitute the basic lesion of this disease. The absence of an exudative reaction and pus formation is the only feature that separates this process from the classic picture of a fulminating septicopyemia. The general histologic features of the bacterial infection and the experimental work by others with artificial infection of sublethally irradiated animals indicates that once the bacteremia is present, the disease progresses extremely rapidly and death results in a short period of time (*11*). Reparative phenomena are lacking in these inflammatory foci, except for the rare occurrence of bacterial phagocytosis.

In swine and in man exposed to total-body irradiation, the bacterial infections observed do not differ significantly from those seen in mice (*14, 15*).

Conclusions

The lesions observed in the intestine probably represent one portal of entry for the bacterial infection at this exposure level.

The pathogenesis of the disease depends on the existence of a bacteremia that rapidly progresses to a fulminating septicopyemia in which purulent exudation is lacking.

References

(*1*) Chrom, S. A.: Studies on the effect of roentgen rays upon the intestinal epithelium and upon the reticulo-endothelial cells of the liver and the spleen. Acta Radiol. 16: 641–660, 1935.

(*2*) Lawrence, J. H., and Tennant, R.: The comparative effects of neutrons and X-rays on the whole body. J. Exper. Med. 66: 667–688, 1937.

(*3*) Miller, C. P., Hammond, C. W., and Tompkins, M.: The role of infection in radiation injury. J. Lab. & Clin. Med. 38: 331–343, 1951.

(*4*) Vogel, H. H., Clark, J. W., Hammond, C. W., Cooper, D. B., and Miller, C. P.: Bacteremia in mice irradiated with fast neutrons and effect of streptomycin on mortality. (Abstract.) Fed. Proc. 13: 445, 1954.

(*5*) Congdon, C. C., Uphoff, D., and Lorenz, E.: Modification of acute irradiation injury in mice and guinea pigs by injection of bone marrow: A histopathologic study. J. Nat. Cancer Inst. 13: 73–107, 1952.

(*6*) WILLIAMS, F. P., and CONGDON, C. C.: IV. Septicemia in irradiated C_3Hb mice and its modification by bone marrow injection. *In* Quart. Rep., Div. Biol. and Med. Res. (Brues, A. M., ed.). Argonne National Laboratory, May, June, July 1952, pp. 17–23.

(*7*) BROWN, J. H., and BRENN, L.: A method for the differential staining of gram-positive and gram-negative bacteria in tissue sections. Bull. John Hopkins Hosp. 48: 69–73, 1931.

(*8*) LORENZ, E., CONGDON, C. C., and UPHOFF, D.: Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. Radiology 58: 863–877, 1952.

(*9*) LORENZ, E., and CONGDON, C. C.: Modification of acute and chronic irradiation injury in experimental animals by injection of bone marrow and tissues originating from hematopoietic organs. Proc. 4th Internat. Congr. Hematol., Mar del Plata, 1952.

(*10*) MOTTRAM, J. C.: Some effects of exposure to radium upon the alimentary canal. Proc. Roy. Soc. Med. (Section of Electro-Therapeutics) 16: 41–44, 1923.

(*11*) HAMMOND, C. W., TOMPKINS, M., and MILLER, C. P.: Studies on susceptibility to infection following ionizing radiation. I. The time of onset and duration of the endogenous bacteremias in mice. J. Exper. Med. 99: 405–410, 1954.

(*12*) HAMMOND, C. W., COLLING, M., COOPER, D. B., and MILLER, C. P.: Studies on susceptibility to infection following ionizing radiation. II. Its estimation by oral inoculation at different times post irradiation. J. Exper. Med. 99: 411–418, 1954.

(*13*) SCHWEINBURG, F. B., SELIGMAN, A. M., and FINE, J.: Transmural migration of intestinal bacteria; a study based on the use of radioactive *Escherichia coli*. New England J. Med. 242: 747–751, 1950.

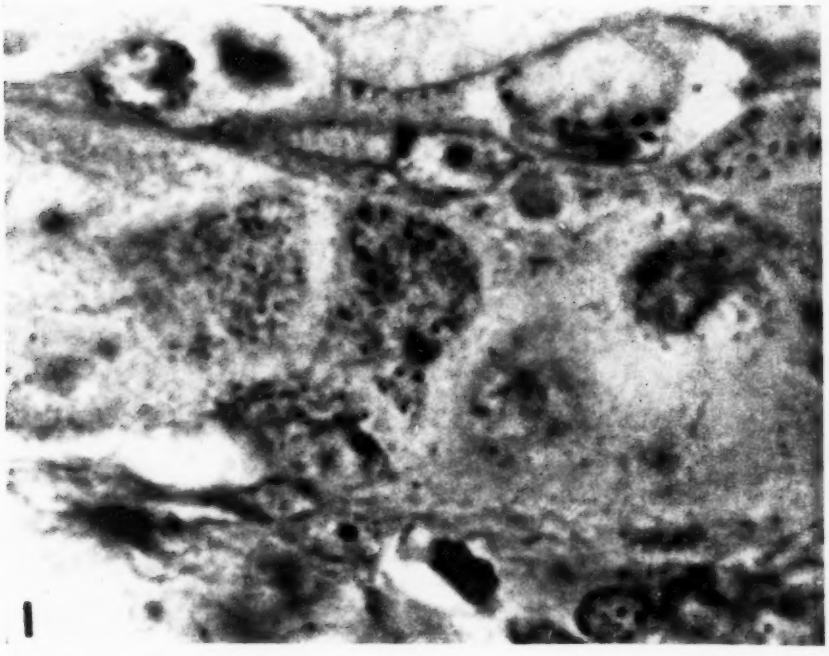
(*14*) TULLIS, J. L.: The response of tissue to total body irradiation. Am. J. Path. 25: 829–851, 1949.

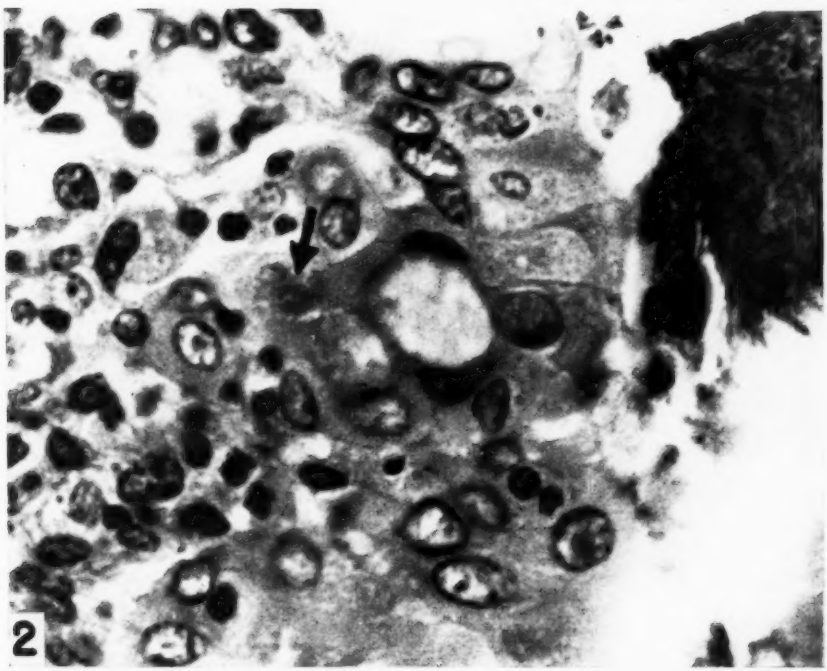
(*15*) LIEBOW, A. A., WARREN, S., and DECOURSEY, E.: Pathology of atomic bomb casualties. Am. J. Path. 25: 853–1027, 1949.

PLATE 51

FIGURE 1.—Scattered bacteria, throughout injured epithelium of the small intestine. Animal killed and autopsied 4 days after 900 r. Brown and Brenn. Oil immersion. × 1,940

FIGURE 2.—Bacterial mass lying against two or three necrotic epithelial cells of the intestine. The stippled material slightly out of focus, in the cytoplasm of the cell containing the large mucous vacuole is bacteria. The tissue to the left of the epithelial surface was an atrophied lymph nodule. Presumably this area would have become a microscopic ulcer. Same animal as figure 1. Hematoxylin and eosin. × 950



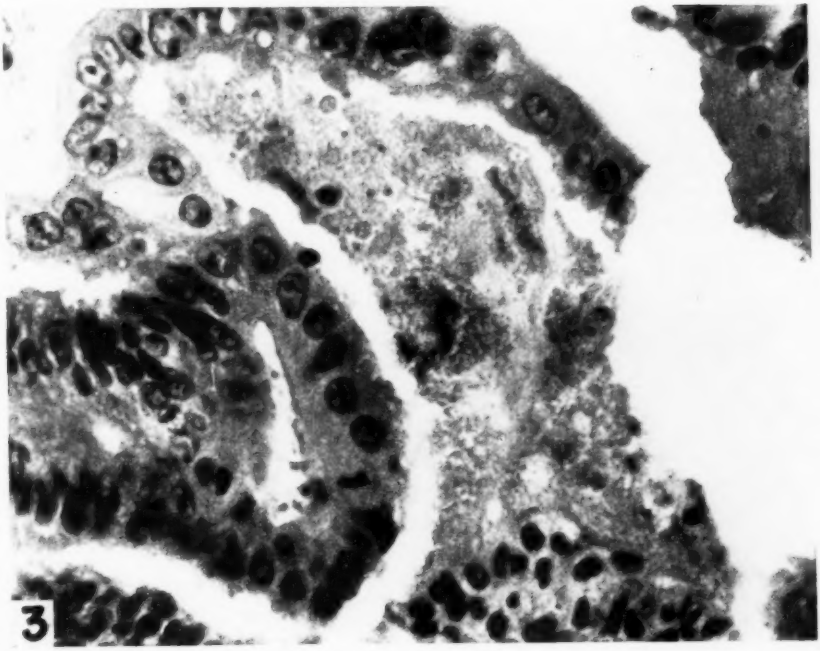


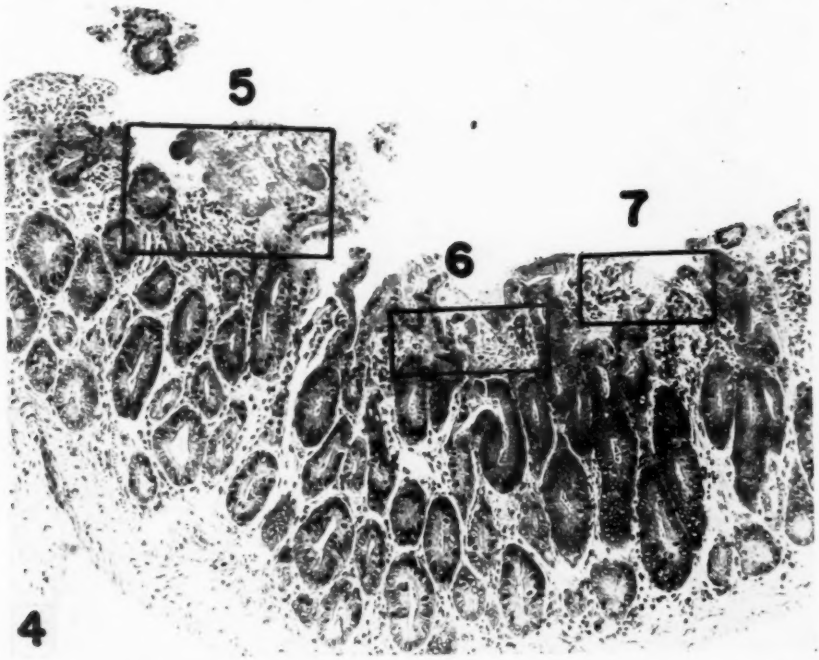
320174—55——5

PLATE 52

FIGURE 3.—Clumps of bacteria in tissue fluid beneath damaged epithelium of the small intestine. The nuclei of the stromal cells are pyknotic. Animal killed and autopsied 4 days after 900 r. Hematoxylin and eosin. × 740

FIGURE 4.—Same animal as figure 3. Low-power view of small intestine showing three separate areas of bacterial invasion. Higher magnifications of these areas are shown in figures 5, 6 and 7. Hematoxylin and eosin. × 165





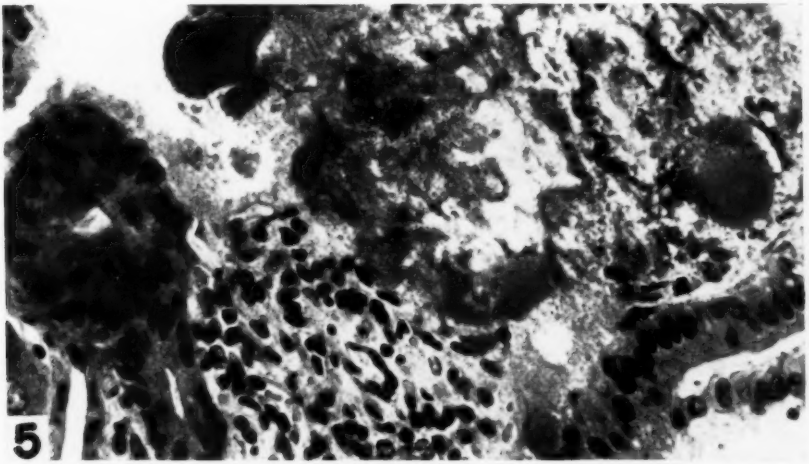
320174—55——6

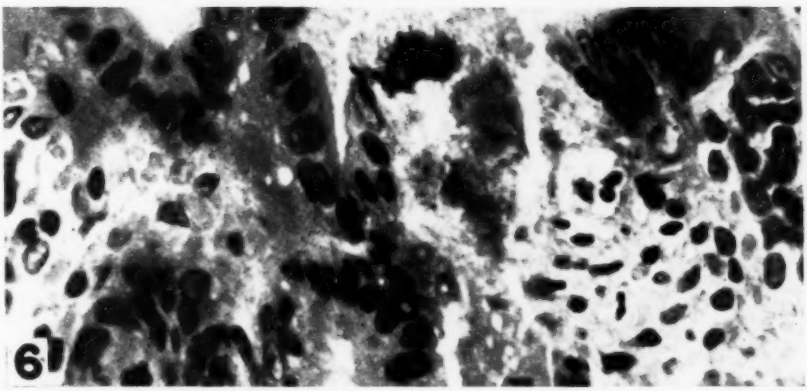
PLATE 53

FIGURE 5.—Bacterial mass lying against necrotic stroma of small intestine. Same animal as figure 3. Hematoxylin and eosin. × 520

FIGURE 6.—Epithelial fissure filled with small bacterial masses. Note necrosis of adjacent stroma. Same animal as figure 3. Hematoxylin and eosin. × 740

FIGURE 7.—Bacteria invading the wall of a necrotic vessel in the stroma of the small intestine. Same animal as figure 3. Hematoxylin and eosin. × 740


5


6

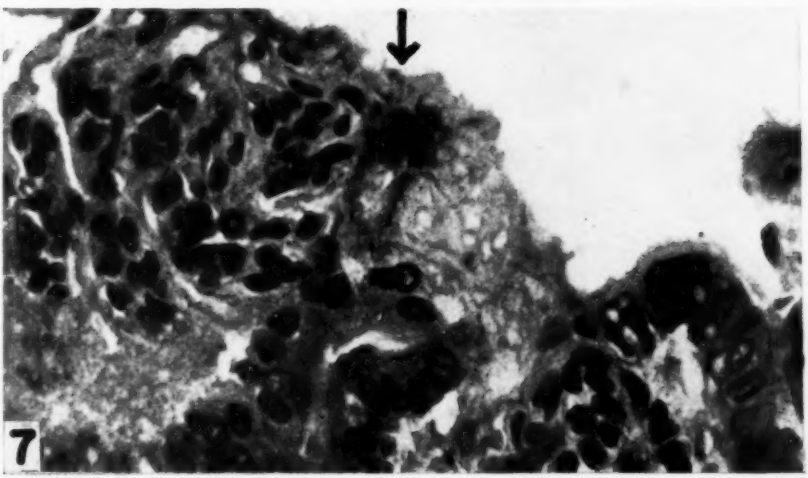
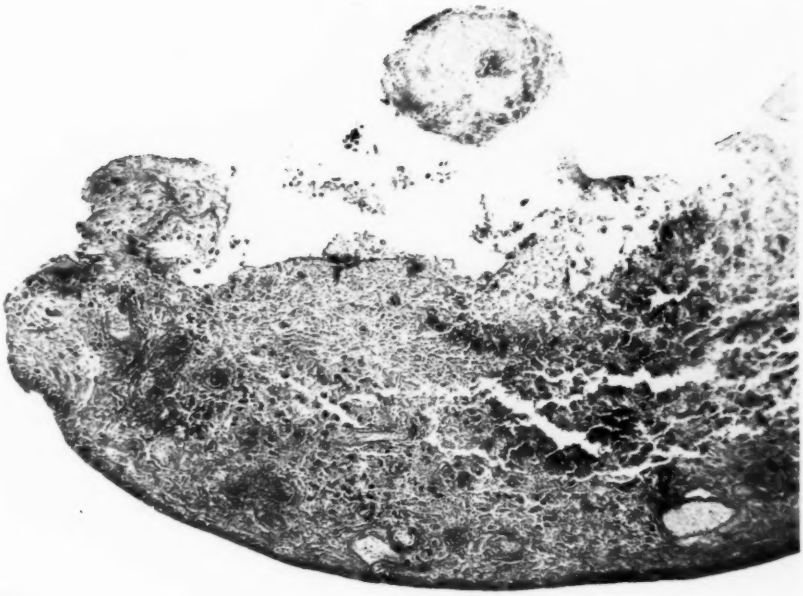

7

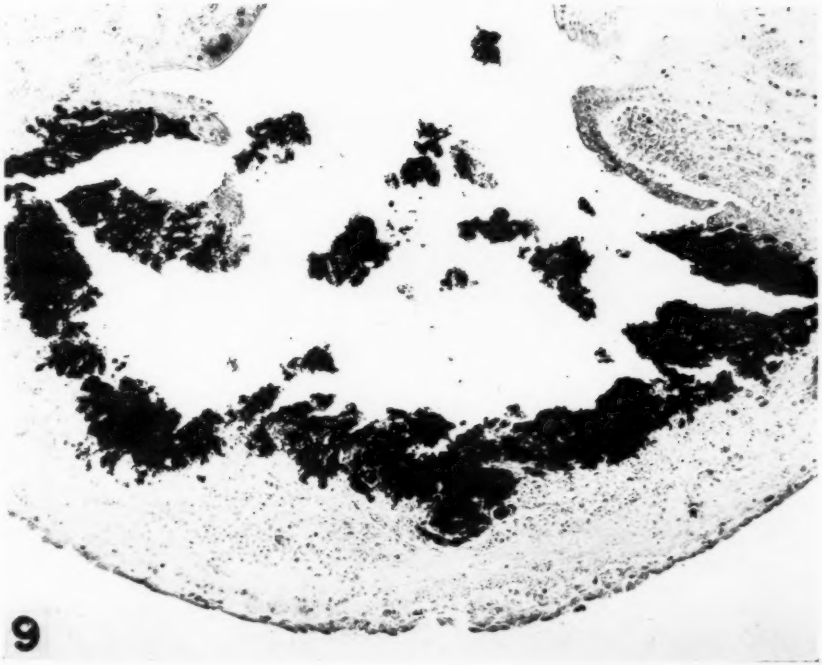
PLATE 54

FIGURE 8.—Diffuse bacterial necrosis of the entire wall of the small intestine at the site of a Peyer's patch. Animal killed and autopsied 4 days after 900 r. Hematoxylin and eosin. × 165

FIGURE 9.—Brown and Brenn stain showing large bacterial ulcer caused by gram-positive organisms at the site of a Peyer's patch. Animal killed and autopsied 8 days after 900 r. × 165



8



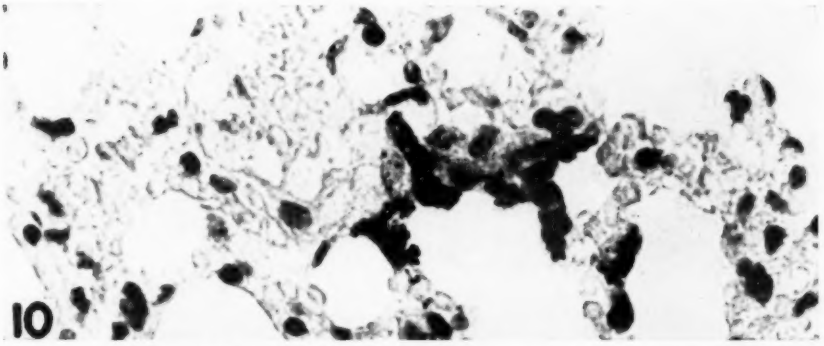
9

PLATE 55

FIGURE 10.—Early lesion showing gram-positive bacteria in lung capillaries. Animal killed and autopsied 10 days after 900 r. Brown and Brenn. × 700

FIGURE 11.—Advanced lesion showing growth of bacteria along vascular channels in the lung. Animal killed and autopsied 9 days after 900 r. Brown and Brenn. × 515

FIGURE 12.—Same animal as figure 11. Low-power view to show widespread bacterial growth in capillaries of the lung. Note the serous exudate in the aleveolar spaces. Hematoxylin and eosin. × 165


10

11

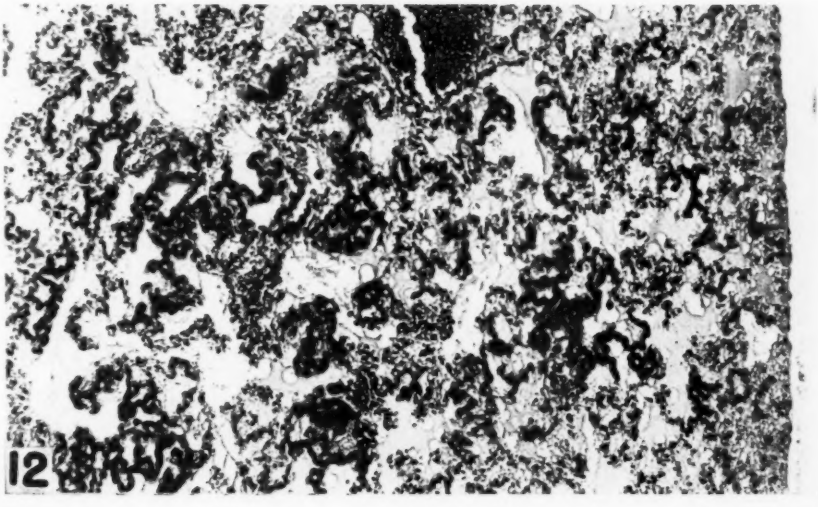
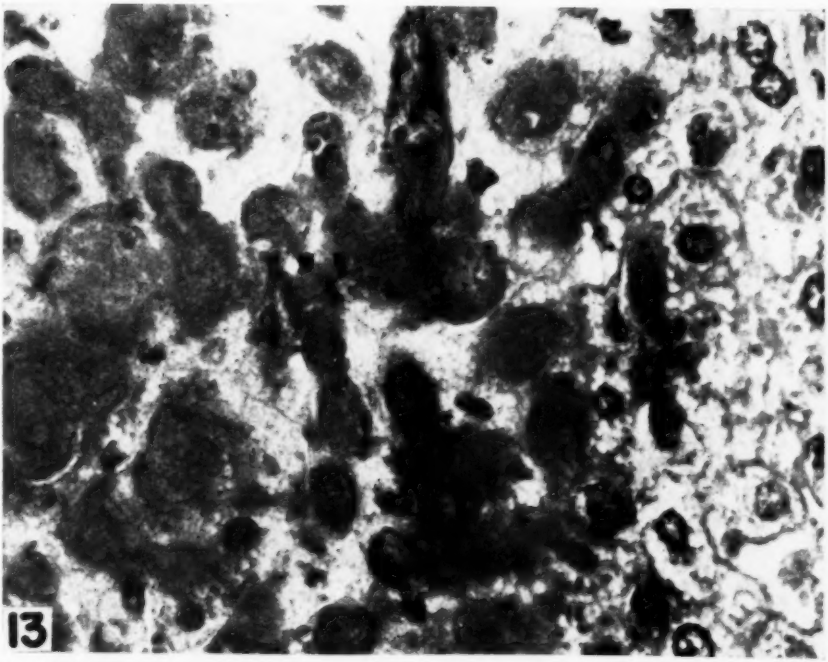
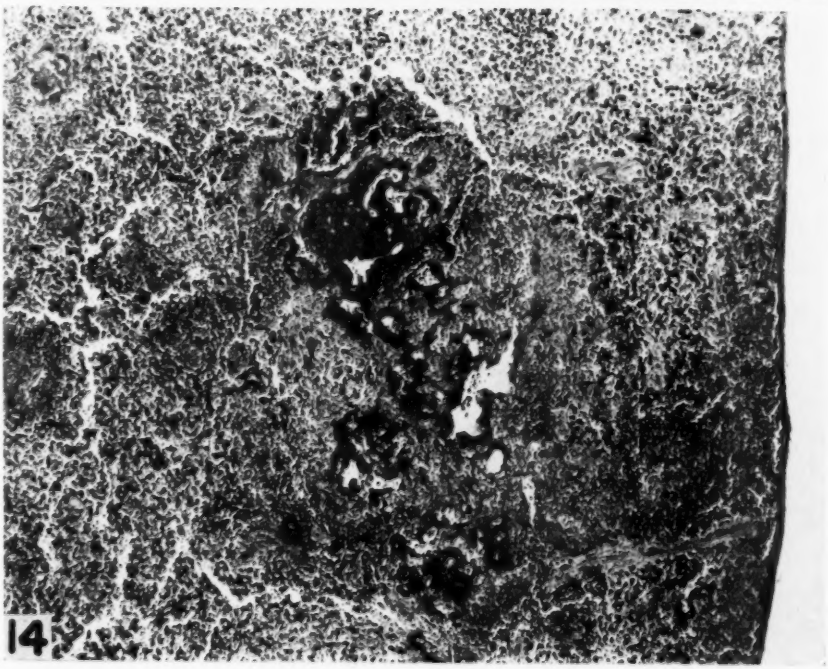

12

PLATE 56

FIGURE 13.—Large area of bacterial necrosis in the liver. Animal was killed and autopsied 10 days after 900 r. Hematoxylin and eosin. × 700

FIGURE 14.—Extensive bacterial necrosis in the spleen. Animal was killed and autopsied 4 days after 900 r. Hematoxylin and eosin. × 165


13


14

Regenerative Growth in the Urodele Forelimb Following Ultraviolet Radiation [1,2]

ELMER G. BUTLER *and* HAROLD F. BLUM,[3] *Department of Biology, Princeton University, Princeton, N. J.*, and *National Cancer Institute,*[4] *Bethesda, Md.*

Effects of ultraviolet radiation on living systems have been studied for over a century, with emphasis in recent years on effects on isolated cell systems. Among effects on organized tissues, *in situ*, is the induction of cancers in rodent skin, introducing problems of growth not apparent in individual cells. The urodele amphibian, which possesses marked capacity for regenerative growth has been the subject of extensive research, but studies of the effects of ultraviolet radiation on such animals have been few. In regeneration and the development of accessory limbs in the salamander, *Amblystoma* (which is the subject of this paper) the problems seem to be quite different from those concerned in isolated cell systems, and to approach closer to those of neoplastic growth.

A brief account of the regenerative process may help to orient the reader with regard to the mechanisms concerned. When a limb is amputated, epidermal healing takes place rapidly, and after this an aggregation of cells, the *regeneration blastema*, is established in the wound area. From the cells which make up the blastema develop the new structures of the regenerate, with the exception of the nerves which grow in from the cut stumps at the amputation level. Many of the factors that underlie the mobilization of cells to establish a regeneration blastema and govern its development are still obscure. It is clear, however, that cells do not migrate from a distance (*1*) and that the blastema results from local transformations of cells at the amputation level. Amputation is commonly regarded as the essential stimulus for establishment of the blastema, although under certain experimental conditions a regeneration blastema may arise long afterward and at a level far proximal to the original level of amputation. Such a situation prevails, for example, in a denervated limb which has undergone extensive regression (*2, 3*), in which case the immediate stimulus for the formation of

[1] Received for publication July 19, 1954.

[2] This investigation was supported in part by a research grant, C 1499, from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

[3] We wish to express our thanks to Doris Manley, Margie R. Matthews, V. R. Gregg, and John S. Cook for technical assistance in carrying out the radiation experiments.

[4] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

the blastema is reinnervation of the limb, particularly the reappearance of nerve fibers at the distal tip.

Under certain experimental conditions two or more complete limbs may form instead of a single one. Of particular interest with regard to the formation of such accessory limbs is the work of Guyénot and his associates (*4*, *5*), who found that experimental deviation of nerves in the adult urodele limb results in the development of a new limb as an outgrowth from the normal one. Depending on experimental conditions, accessory structures vary from duplication of digits and other portions of the hand, to the establishment of a supernumerary arm near the shoulder. Accessory limbs may be formed as a result of other procedures, Nassonov (*6*) having obtained them in the axolotl by simply tying a ligature around the limb. Brunst (*7*), by localized X radiation of the lumbosacral region of the axolotl produced complete supernumerary hind limbs articulating with the pelvis. More recently, Breedis (*8*) found a high incidence of accessory limbs in adult *Triturus* at the site of injection of carcinogenic substances. In this laboratory Rieck (*9*), whose paper may be consulted for references to earlier literature, has found accessory hands and digits after subjecting the limb bud of *Amblystoma* larvae to ultraviolet radiation.

The present studies concern specifically the formation of blastemata and accessory limb structures in the salamander after localized irradiation with ultraviolet of areas of the larval limb, and of regression and photorecovery after such radiation. A detailed description of the anatomical characteristics of the accessory limb structures will not be undertaken here, but will be the subject of a later communication elsewhere.

Materials and Methods

Larvae of *Amblystoma opacum* and *Amblystoma punctatum* were used in these studies. Eggs and young larvae were obtained from North Carolina or from the Princeton area and reared in the laboratory. In each experiment the larvae were staged with respect to age, using Harrison's stages for the younger larvae (see *10*) and total length as an index of age of older animals.

Ultraviolet radiation was applied in the following manner: A larva was anesthetized with a 1:5,000 solution of MS 222 and then transferred to a 1:15,000 solution for the period of exposure. Each animal was placed on its back, either on absorbent cotton or black nylon fiber, in a petri dish with just enough anesthetic solution to cover it. The right forelimb was extended as far as possible and oriented so that it was approximately at right angles to the body wall, the flexor surface of the forearm uppermost. Using thin pieces of aluminum foil as shielding material, the entire animal was covered except for a small area of the right limb. The extent and location of the unshielded area that received the radiation will be given in connection with the experiments discussed later. The left limb, completely shielded from the ultraviolet radiation, served as a control. Unless the whole surface of the petri dish is shielded, enough ultraviolet radiation may be scattered by reflection from the

cotton fibers to injure or kill a larva. This was a source of error in some of our earlier experiments. Later, black nylon fiber was used in place of cotton, in order to reduce reflection of ultraviolet radiation passing through the aperture of the shield but not impinging directly upon the limb.

Preparation of the animal for irradiation was carried out under a dissecting microscope equipped with a movable stage so designed that it could be moved under the source of radiation with a minimum of disturbance (*9*). Measured doses of polychromatic mercury-arc radiation were obtained by a method which was essentially that of Blum, Kirby-Smith, and Grady (*11*, and see *9*). The dosage employed in all experiments was 1.9×10^8 ergs per square centimeter of radiant energy of wavelengths 0.31 μ and shorter, at an intensity of 1.55×10^5 ergs per square centimeter per second; (longer wavelengths are present but ineffective in producing these results). This is a high dosage compared with the individual repeated doses necessary to produce cutaneous cancer in mice (*12*) or single doses required to kill microorganisms.

Each larva received a single dose of ultraviolet radiation. Afterward the larva was kept in an incubator at 20° ± 0.5° C. In most cases the interior of the incubator was dark, but in experiments concerned with photorecovery the interior was illuminated with banks of "fluorescent" lamps (Daylite type). In some of the later experiments the animals were kept in the light for a few days only, then transferred to the dark, since photorecovery reaches its full extent in a relatively short time; the experiments of Blum and Matthews (*13*) indicate that photorecovery is virtually complete in *Amblystoma* in 48 hours, but not in 24. The animals were removed from the incubators for short periods for observation and three times a week for feeding. The diet was *Daphnia*, *Artemia*, small *Tubifex* and enchytrae.

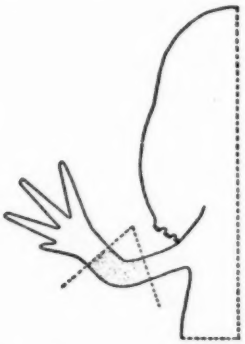
At the termination of each experiment the animals were fixed in Bouin's fluid or in formol-alcohol fixative. The latter was used where it was desired to stain *in toto* with methylene blue, in preparing total transparent mounts for study of the skeletal elements of a regenerate limb.

Results

Irradiation of the Complete Elbow Region

The elbow region of 150 *A. opacum* larvae, 20 to 26 mm. in total length, was exposed to ultraviolet radiation. As indicated in text-figure 1, the shield was so placed that in all cases the elbow joint was completely exposed, including a portion of the distal region of the upper arm and proximal region of the forearm. The amount of the upper arm and forearm which was exposed varied with the length of the arm. Approximately half of the animals at the time of irradiation (total length, 20 to 22 mm.) possessed limbs in which the hand was either in the three-digit stage of development, or in which the fourth digit was just appearing; the other half of the animals (total length, 23 to 26 mm.) possessed limbs in which

the hand had reached the definitive four-digit condition. All animals in this experiment were kept in the dark after irradiation, except for brief periods of observation. Of the total of 150 larvae irradiated, 110 developed accessory growths of the forelimb; these 110 also clearly exhibited a certain amount of limb regression, particularly of the forearm; 16 developed no accessory growths but showed regression; 4 appeared to be unaffected by the radiation; 20 animals died so soon after irradiation that no alterations of the limb could be observed.



TEXT-FIGURE 1.—Irradiation of the complete elbow region of right forelimb. Animal lies on its back. Stippled area represents region exposed to radiation; remainder of animal was shielded.

Initial visible effects of the radiation include a roughening and loosening of the skin in the unshielded irradiated area, with the result that it is thrown into ridges and sloughed off within the first 24 hours. This is shown in figure 1. At about the same time, stasis becomes evident in the blood vessels of the general distal area, especially pronounced in the digits, although these were not exposed to the radiation. Although there is much individual variation with respect to the time when the cutaneous and vascular changes first appear, they are ordinarily evident within 2 to 4 days after irradiation. The loosened skin is gradually replaced by new skin, usually within the first two weeks, sometimes sooner.

Stasis in the blood vessels of the digits and the general forearm region gradually subsides during the first two weeks. During the second week additional alterations consisting of partial fusion of digits and digital regression frequently appear. Whether these changes are causally related to the previous condition of stasis has not been determined. Extensive regression of the digits does not always occur, but is frequently evident, and results in the hand becoming club-shaped, with only the proximal stubs of the digits remaining. This condition is transient and scarcely have the digits regressed to their bases when they begin to regenerate and are rapidly reconstituted. By the end of the third week, postirradiation, the hand has returned to a normal appearance.

During the third week an important regressive change becomes evident in the forearm proximal to the carpus. The regression gradually becomes

very extensive and it seems doubtful that it is associated with the transient digital regression just described. Grossly, this second regressive activity involves a gradual but persistent shortening of both the humerus and the radio-ulnar region. In one experiment with *A. punctatum*, measurements on 32 surviving animals out of 163 kept in the dark, showed 32 percent reduction in length of the forearm and 26 percent in length of the humerus, as compared with the contralateral unexposed arm (table 1). The wrist, so to speak, "retreats" toward the elbow. Although we are dealing with growing larvae, in which the limb normally should be increasing in length, it is not to be concluded that the shortening of the forearm is simply a result of arrested growth in this region. Rather, as measurements show, it is the result of active regression. Two essentially antagonistic processes are at work: normal growth in length is taking place in the unirradiated proximal and distal ends of the limb; regression and consequent shortening are occurring in the radio-ulnar region, and to a lesser extent in the irradiated region of the upper arm just proximal to the elbow joint. As a result of these activities, although the proximal region of the limb and the general region of the hand appear normal at the end of 3 or 4 weeks, the forearm is markedly reduced in length and is often misshapen and bent.

During the fourth and fifth weeks a new type of activity begins in the irradiated region. It is during this period that a protrusion may appear near the elbow or along the posterior border of the forearm. In its manner of development and in the potentialities which it later manifests, this protrusion resembles a *regeneration blastema* in every way, and it will be so referred to from now on.

The precise point on the limb at which the blastema first appears varies among individuals. On some limbs it is nearer the elbow, on others nearer the wrist. As a result of regression of the forearm, the wrist and the elbow are much closer than normal, making it difficult to state whether a blastema appears near the elbow or near the wrist.

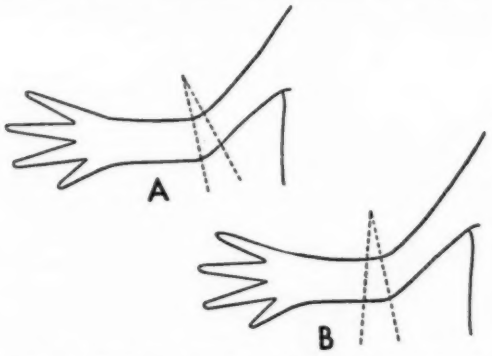
Once a blastema is established it grows rapidly in size and soon begins to exhibit morphogenetic capacities. Differentiation takes place within the blastema and one or more digits appear. Figures 2 to 4 show a group of representative cases of these accessory growths. No attempt will be made at this time to discuss in detail the morphogenetic pattern which the accessory growths assume. The photographs of the various limbs were taken at the termination of observation of each case concerned. At this time there was no evidence that the accessory structure would undergo any further morphological change—the blastema had apparently exhausted its complement of potentialities. Some of these superregenerates have been studied in sections, and also as transparent mounts stained with methylene blue so that the skeletal elements are clearly shown. Examination of the limb shown in figure 4, for example, reveals that within the superregenerate there are extensions of the radius and ulna, a set of carpals, and a skeletal mass which can be regarded as fused metacarpals. Each digit possesses three distinct phalangeal elements.

Classification of the various types of regenerates on the basis of the number of digits or other morphological characteristics is arbitrary and of little

significance. However, considering the 110 cases that developed accessory growths as referred to above, we have assigned the following percentages: single spikelike outgrowth, 51 percent; 2 digits, 33 percent; 3 digits, 12 percent; others, 4 percent.

Irradiation of Areas of the Limb Other Than the Complete Elbow Joint

Primarily because previous investigations had shown that regenerative activity is normally preceded by pronounced cellular alterations in formed structures of the limb, and that in the case of induction of regeneration without amputation these changes were more likely to occur at the terminal ends of limb segments, it seemed desirable to begin our experiments



TEXT-FIGURE 2.—*A*) Irradiation of area immediately proximal to elbow joint. *B*) Irradiation of area immediately distal to elbow joint. In each case the sector within the broken lines represents the region exposed to radiation; remainder of the animal was shielded.

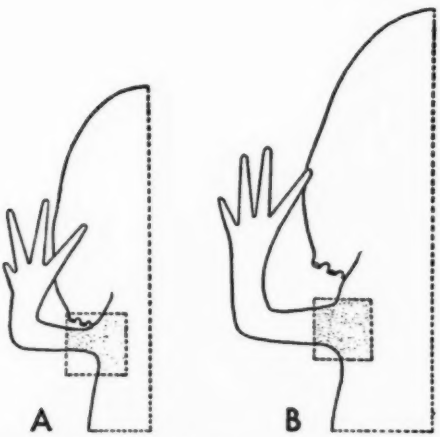
with localized irradiation of the elbow. As has been noted, the accessory growths that took place occurred either at the elbow, or after regression of the forearm, at the wrist. The question arises as to whether exposure of a small area to radiation either immediately proximal or distal to the elbow joint will result in regenerative growth. Also, will growth result from localized irradiation of the shoulder region?

Irradiation immediately distal or proximal to the elbow joint.—Since the results of irradiation either immediately above or below the elbow were essentially the same, they will be dealt with together. Shielding was carried out as shown in text-figure 2, *A* and *B*. The dosage was the same as in the previous experiment and all animals were kept in the dark after irradiation. A total of 115 animals was irradiated: 55 above the elbow; 60 below the elbow. In none of these did any accessory growths occur, although they were observed for a period of over 10 weeks. No indication of formation of a blastema was observed.

Regression was clearly evident in the irradiated and in adjacent areas. In animals irradiated above the elbow, regression was limited largely to the distal end of the upper arm. Measurements on the 29 surviving animals showed 21 percent reduction in length of the upper arm, with only 2 per-

cent reduction in length of the forearm. In some cases the regression was extreme, making the elbow and shoulder joints so close to one another at the end of the experiment that the limb appeared to consist primarily of a forearm. Correspondingly, after application of radiation below the elbow joint, pronounced regression of the forearm occurred. Measurements on 34 surviving animals showed 15 percent shortening of the forearm with 2.5 percent shortening of the upper arm.

It is clear from these results that regression without regenerative activity may result from the effects of ultraviolet radiation, but we are not prepared at this time to offer an explanation of the contrasting results of total



TEXT-FIGURE 3.—Irradiation of shoulder area. Aperture in shield was 1-mm. square. *A*) Larvae, total length 20 to 23 mm. *B*) Larvae, total length 24 to 26 mm. In each case the stippled area represents the region exposed to radiation; remainder of the animal was shielded.

and partial irradiation of the elbow. Dosage and extent of damage may be important factors.

Irradiation of the shoulder area.—*A. opacum* larvae, total length 20 mm. to 26 mm., were used and shielded as shown in text-figure 3, *A* and *B*. As the figure shows, in the smaller animals with shorter limbs the irradiated area included, in addition to the shoulder joint, about the proximal half of the humerus. By the time the animals had reached 24-mm. total length, the forelimb had grown to the extent that the irradiated area included not more than the proximal one quarter of the humerus. As in previous experiments, the animals were kept in the dark after irradiation.

Of 170 larval limbs irradiated, accessory growths appeared in only two cases. These are shown in figures 5 and 6. Each of these growths was on one of the younger larvae of the group; one (NS-117, fig. 5) was 21-mm. total length; the other (NS-76, fig. 6) was 22-mm. total length when irradiated. The other 168 animals, although observed for varying periods up to 82 days, showed no signs of the establishment of blastemata or regen-

erates. In accordance with the results of previous experiments, however, regression of the proximal end of the upper arm was clearly evident.

It is interesting that in the two cases in which regenerative growth occurred, the structures formed were morphologically more extensive than in the case of elbow or wrist regenerates. Each of the superregenerates shown in figures 5 and 6 possesses an upper arm, forearm, wrist, and digits. After irradiation of the elbow, regenerates consisting of forearm, wrist, and digits develop; and only digital structures develop from the carpal region. These observations resemble those of Guyénot and his associates (*4*, *5*) in experiments on inducing regeneration by deviation of nerves.

Photorecovery

The phenomenon of photorecovery, or photoreactivation, consists of the repair of certain types of damage caused by the action of ultraviolet radiation on living systems, by subsequent exposure to light of wavelengths in the approximate range 0.3 to 0.5 μ, which will be referred to somewhat inaccurately in this paper as "visible" light. Since its description by Kelner and by Dulbecco in 1949, photorecovery has been described in a variety of plants and animals including the larvae of *Amblystoma* (*13*).

In the present studies illumination with "visible" light following exposure to ultraviolet radiation of the elbow region results in a marked decrease in the extent of regression. This is shown in table 1 which presents results from one experiment in which 326 larvae of *A. punctatum* were exposed in pairs to ultraviolet radiation. After exposure, one animal from each pair was placed in the dark, the other in the light. The elbow region of one arm was exposed to the radiation as indicated in figure 1, the other arm served as a control. In only 32 cases did both members of the pair survive. Among these, as shown in the table, both the upper arm and forearm of the exposed limb was short as compared with the shielded control limb—the shortening being much greater in the animals kept in the dark than in those kept in the light. The high mortality in this experiment resulted from scattered radiation because of improper shielding. The "unexposed" limb probably received a small amount of radiation, but the results are, nevertheless, valid comparatively. Survival among the animals kept in the light (67 percent) was much greater than among those kept in the dark (29 percent). These results parallel previous observations (*13*) that larvae subjected to whole-body radiation, consisting of repeated doses much smaller than those used in these studies, survived better in the light than in the dark. Animals that had not been subjected to ultraviolet radiation survived equally as well in the dark as in the light.

In the experiment just described it was not possible, because of the high mortality, to establish whether the incidence of accessory limbs is affected by illumination subsequent to exposure to ultraviolet radiation, but in a current experiment not yet completed, it is clear that the devel-

opment of accessory structures is much reduced by placing the animals in light for a few days after the exposure. Thus, photorecovery reduces regression and formation of accessory structures in a parallel manner.

TABLE 1.—*Regression of limbs of larvae of* A. punctatum *after exposure of the elbow region to ultraviolet radiation. (For details regarding number of animals used see text)*

		Average length of upper arm (*mm.*)	Shortening upper arm (*percent*)	Average length of forearm (*mm.*)	Shortening forearm (*percent*)
LIGHT	control arm	2. 20	.....	2. 48	.....
	exposed arm	2. 11	4. 0	2. 39	4. 0
DARK	control arm	2. 08	.....	2. 42	.....
	exposed arm	1. 57	32. 0	1. 92	26. 0

Discussion

Ultraviolet radiation has a number of effects on living cells, involving more than one type of light-absorbing molecule. Some of these effects are subject to photorecovery, others are not (*14*). In the present experiments it is to be supposed that there have been various effects on the cells of the area exposed to ultraviolet radiation, particularly in view of the high doses used. It seems likely that some cells were killed by direct cytolysis. Some of Rieck's histological observations (*9*) on the limb buds of *Amblystoma* suggest direct lysis, and it seems likely that this factor is concerned to some extent in the regression we have observed. The high degree of photorecovery we have found indicates that lysis is a relatively small factor in the total regression, since direct lysis as observed in other types of cells is not subject to photorecovery (*14*). The killing of bacteria and fungi with relatively low doses of ultraviolet radiation is to a high degree reversible by visible light. It seems probable that some such photorecoverable lethal effect is concerned in regression of the urodele limb that has been subjected to ultraviolet radiation. Perhaps this is the major factor.

The most obvious immediate effect of ultraviolet radiation on a variety of cells is the slowing of cell division. This effect is subject to photorecovery, in the sense that the rate of cell division is more rapidly returned to normal by exposure to visible light. Regression involves active destruction of cells; regeneration, active proliferation of cells. Hence, the rate and extent of regression might be thought of for analytical purposes as an algebraic summation of these two opposing activities. Thus, the effect of visible light in inhibiting regression might be explained in part by an increase in proliferation relative to destruction of cells. But the return to normal rate of cell division by photorecovery is a relatively transitory process that could hardly account for all the differences in regression in light and dark that are indicated in table 1.

In the establishment of blastemata and the growth of accessory limbs, there is obviously a temporary increase in the rate of cell division beyond the rate normally prevailing in the limb just before it was subjected to the radiation. The evidence from studies on isolated cell systems, including dividing eggs, points to the conclusion that ultraviolet radiation does not stimulate cell division beyond normal rates in such systems. It seems probable that the increase in the rate of cell division found in the case of the regenerating urodele limb, in the hyperplasia of epidermis after sunburn, and in experimentally induced cancer of the skin, results from indirect action of the ultraviolet radiation on the tissue rather than directly on the individual cells.

Whatever the basic effects of ultraviolet radiation on the urodele limb, it is clear that the subsequent regression and regeneration depend upon morphogenetic capacities characteristic of the species. To what extent the action of ultraviolet radiation on the cells may modify the subsequent changes remains to be determined. It is possible that the radiation only accomplishes, in essence, the same result as amputation of a limb, which in the urodele is followed by transitory regression and later regeneration. Is there a definitive relationship between these two processes in that regression is an essential precursor of regeneration? In the present studies, irradiation of the whole elbow region has been followed by regression and regeneration, whereas irradiation of a part of the elbow region has been followed by regression only. Whether this is dependent upon the area, the extent of structural alteration involved, or other factors, we are not able to answer at present. It seems evident that ultraviolet radiation, combined with photorecovery, provides a method for the study of numerous problems of developmental growth.

Summary

Localized ultraviolet irradiation of regions of the forelimb of larval *Amblystoma* may result in two major changes: regression of the limb; and the induction of an accessory limb structure at the point irradiated. The incidence and morphological characteristics of accessory structures vary according to the site of exposure. The extent of regression and the incidence of accessory structures are subject to photorecovery, both being markedly reduced by illumination with "visible" light for a few days after exposure to ultraviolet radiation.

References

(*1*) Butler, E. G., and O'Brien, J. P.: Effects of localized x-radiation on regeneration of the urodele limb. Anat. Rec. 48: 407–413, 1942.

(*2*) Schotté, O. E., and Butler, E. G.: Morphological effects of denervation and amputation of limbs in urodele larvae. J. Exper. Zool. 87: 279–322, 1941.

(*3*) Butler, E. G., and Schotté, O. E.: Histological alterations in denervated non-regenerating limbs of urodele larvae. J. Exper. Zool. 88: 307–341, 1941.

(*4*) Guyénot, E., Dinichert-Favarger, J., and Galland, M.: L'exploration du territoire de la patte antérieure du Triton. Rev. suisse Zool. 55 (suppl. 2): 1–120, 1948.

(5) KIORTSIS, V.: Potentialités du territoire patte chez le Triton. Rev. suisse Zool. 60: 301–410, 1953.

(6) NASSONOV, N. V.: Die regeneration der Axolotlextremitäten nach Ligaturanlegung. Arch. f. Entw.-mech.d. Organ. 121: 639–657, 1930.

(7) BRUNST, V. V.: Influence of local x-ray treatment on the development of extremities of the young axolotl (Siredon mexicanum). J. Exper. Zool. 114: 1–49, 1950.

(8) BREEDIS, C.: Induction of accessory limbs and of sarcoma in the newt (*Triturus viridescens*) with carcinogenic substances. Cancer Res. 12: 861–866, 1952.

(9) RIECK, A. F.: The effects of ultraviolet, and of photorecovery, on the developing forelimb of *Amblystoma*. J. Morphol. 94: 367–408, 1954.

(10) HAMBURGER, V.: A Manual of Experimental Embryology. Chicago, Univ. of Chicago Press, 1942.

(11) BLUM, H. F., KIRBY-SMITH, J. S., and GRADY, H. G.: Quantitative induction of tumors in mice with ultraviolet radiation. J. Nat. Cancer Inst. 2: 259–268, 1941.

(12) BLUM, H. F.: On the mechanism of cancer induction by ultraviolet radiation. J. Nat. Cancer Inst. 11: 463–495, 1950.

(13) BLUM, H. F., and MATTHEWS, M. R.: Photorecovery from the effects of ultraviolet radiation in salamander larvae. J. Cell. & Comp. Physiol. 39: 57–72, 1952.

(14) BLUM, H. F., COOK, J. S., and LOOS, G. M.: A comparison of five effects of ultraviolet light on the Arbacia egg. J. Gen. Physiol. 37: 313–324, 1954.

PLATE 57

FIGURE 1.—*Amblystoma* larva (X-2), 24-mm. total length, irradiation of complete elbow of right forelimb (see text-fig. 1). Ventral aspect photographed one-half hour after irradiation. Note loosened skin, which clearly shows extent of area irradiated.

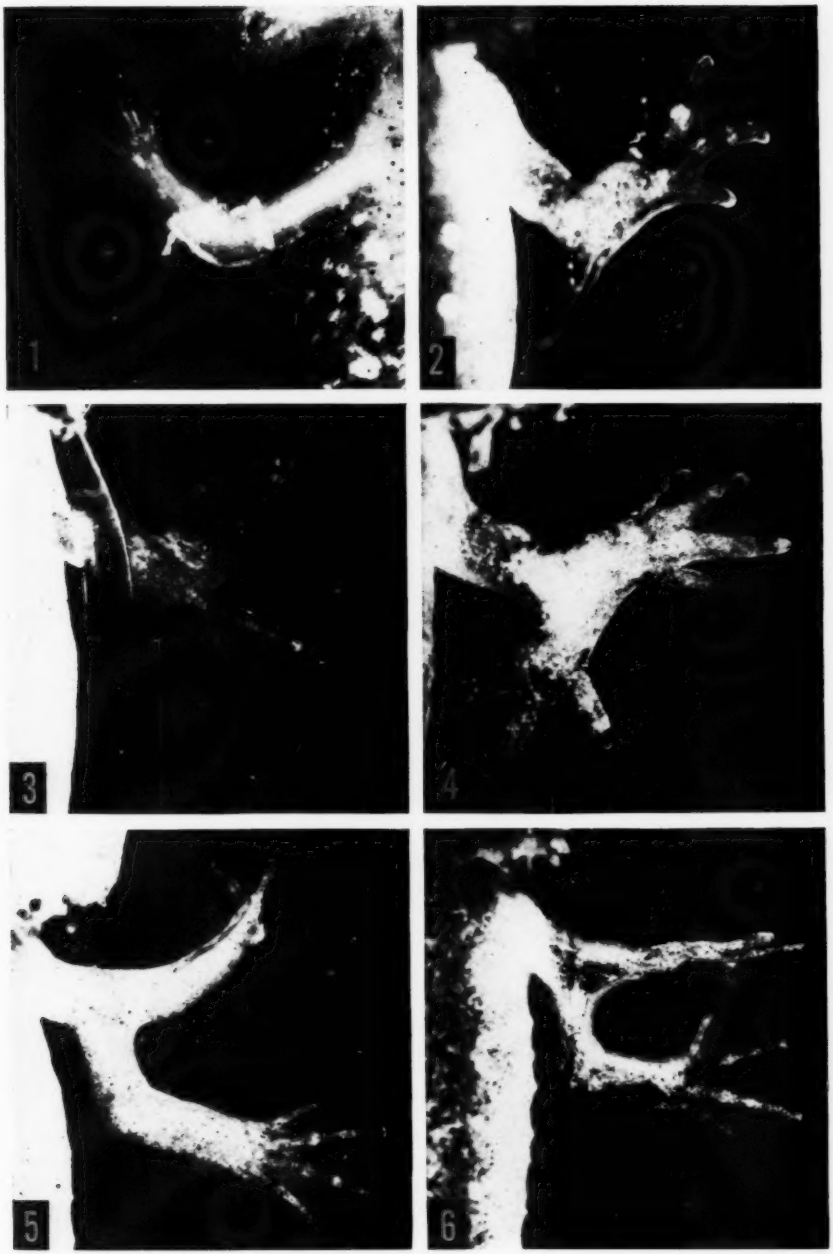
FIGURE 2.—*Amblystoma opacum* larva (OD–132), right forelimb, dorsal aspect. A single digit-like accessory growth at elbow. Photographed 98 days postirradiation.

FIGURE 3.—*Amblystoma opacum* larva (OD-88), right forelimb, dorsal aspect. Two accessory digits at the wrist (*normal* four digits above; *accessory* digits below). Photographed 107 days postirradiation.

FIGURE 4.—*Amblystoma opacum* larva (OD-137), right forelimb, dorsal aspect. An accessory hand with three digits at the elbow (*normal* four-digit hand above; *accessory* three-digit hand below). Photographed 107 days postirradiation.

FIGURE 5.—*Amblystoma opacum* larva (NS-117), right forelimb, dorsal aspect. An accessory limb attached to normal forelimb distal to head of humerus possesses two digits (*accessory* limb above; *normal* forelimb below). Photographed 81 days post-irradiation.

FIGURE 6.—*Amblystoma opacum* larva (NS-76), right forelimb, dorsal aspect. An accessory limb attached to normal forelimb distal to head of humerus possesses three digits (*accessory* limb above; *normal* forelimb below). Photographed 75 days postirradiation.



320174—55——8

The Effect of Fractionated X-Ray Dosage Upon the Mouse Testis. I. Maximum Weight Loss Following 80 to 240 r Given in 2 to 5 Fractions During 1 to 4 Days [1]

HENRY I. KOHN *and* ROBERT F. KALLMAN,[2]
Radiological Laboratory,[3] Department of Radiology, University of California School of Medicine, San Francisco, Calif.

In the case of those organs commonly exposed to X rays during radiation therapy, it is generally held that a given dose of radiation produces less immediate damage when fractionated than when undivided. Early experimenters, however, concluded that fractionation might increase the injury sustained by the male germinal epithelium (*1–3*). Since such a difference in response to fractionation suggests a difference in the mechanism whereby damage is produced in the tissues, it was of interest to reinvestigate the effect of fractionation upon testicular injury.

Changes in testicular weight provided the basis for a simple quantitative method of measuring radiation injury. Dramatic changes in testicular weight had been noted by the earliest observers (*4, 5*), and were plotted as a function of time for single organs by von Wattenwyl and Joël (*6*). But it was in Lorenz' laboratory at the National Cancer Institute that testicular weight was first used extensively by Lorenz and Eschenbrenner as a quantitative measure of radiologic damage (*7, 8*).

Present use of the method rests upon three major premises, experimentally demonstrated for the mouse, and probably also true with slight modifications (*9*) for the rat and hamster. *a*) Testicular weight loss is maximum at about 4 weeks following doses up to 1,000 r or more (*9, 10*). *b*) Weight loss depends only upon the dose to the testes and is an exponential function of that dose (*9*). *c*) Weight loss is due primarily to losses in the germinal epithelium and correlates well with the histologic changes. The changes in weight of the interstitial tissue are trivial (*8, 10*).

The studies reported here involved the acute reaction of the testes of the mouse at approximately 4 weeks after irradiation. Fractionation of dose neither increased nor decreased this reaction. The relation of the findings to previous ones, including Lorenz' work on chronic exposure

[1] Received for publication September 24, 1954.
[2] The authors gratefully acknowledge the technical assistance of Mr. Vernon E. Wilder and Miss Ruth T. Connelly.
[3] The Radiological Laboratory is one of the research and development installations of the U. S. Atomic Energy Commission.

and to current concepts of spermatogenesis, will be dealt with in the discussion.

Materials and Methods

Mice.—The CAF_1 mice were the F_1 generation from the cross (BALB/c) ♀ × (A/He) ♂, 115 to 190 days of age. Four or sometimes three animals were housed in drawer-type cages with wire mesh floors 130 $cm.^2$ in area, constantly supplied with Purina laboratory chow checkers and water. Fighting leading to serious injury did not occur. No animal died from any cause during the experiments.

In setting up an experiment, animals weighing less than 25 or more than 34 gm. were always discarded, the usual limits being 26 and 33 gm. The animals were distributed so that the mean weight and the distribution of weights in all experimental groups were alike.

This procedure was based upon the finding in mature animals, selected as above, that testicular weight was closely correlated with body weight but not with age (*9*). The correlation was re-examined using the data for all controls of the past 2 years, including those of experiments lasting up to 6 months. Of 171 control animals, 3 had but one testis, and 6 others had pairs weighing less than 180 mg. These 9 animals were discarded. For the remaining 162 animals, body weight and testicular weight (± standard deviation) were as follows:

Body weight (gm.)	*Number of animals*	*Testicular weight (mg.)*
25–26	5	210.0 ± 12.3
26–28	33	217.0 ± 15.7
28–30	71	226.5 ± 14.0
30–32	35	230.3 ± 15.7
32–34	18	237.8 ± 18.1

The regression of testicular weight T (mg.) on body weight B (gm.) for the five classes of body weights listed was

$$T = 129 + 3.31\,B \pm 1.7,$$

confirming the previous finding. It was also noted that the data for body weight and testicular weight appeared to be normally distributed.

The data for age and testicular weight were as follows:

Age (days)	*Number of animals*	*Testicular weight (mg.)*
110–129	6	227 ± 12.7
130–149	46	228 ± 17.0
150–169	47	227 ± 15.5
170–189	11	226 ± 14.8
190–209	25	230 ± 14.5
210–229	22	236 ± 18.2
290–309	4	237 ± 15.2

Technique of irradiation.—The animals were irradiated as described previously (*9*). Total-body exposure was employed under conditions developing maximum scatter. The X-ray beam from a 250 kvc unit

was filtered to HVL = 1.55 mm. Cu. The tissue dose-rate was 33 to 45 r per minute.

Weighing of testes.—The animals were decapitated. The testes, freed of the epididymides, were blotted and weighed immediately after removal. To determine the dry weight, the left testis was placed in a tared aluminum cup and dried at 92° C. for 20 to 24 hours. Before completion of cooling to room temperature, the cup was placed in a closed dish, since it was found that the dried organ gained weight when exposed to the air at room temperature.

The left and right testes, on the average, were practically equal in weight. In 111 animals, the left testis averaged 50.5 and the right testis, 49.5 percent of the total fresh weight. The average for groups of 5 to 10 animals differed by 1 to 2 percent.

The fresh weights always refer to the pair of testes and are in milligrams. The dry weights are expressed as percentages of the fresh weights.

Two criteria were employed for the rejection of a weighing: *a*) obvious biological abnormality, *e. g.* only one testis present; *b*) when rejection of that weighing would decrease the coefficient of variation for the group at least 33 percent, the value of the coefficient initially having been greater than 0.1. Criterion *a* was the more frequently employed. In the four experiments of table 1, set up with 301 animals, 17 animals were rejected.

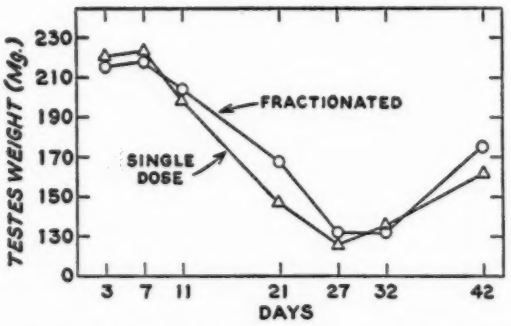
TABLE 1.—*Minimum testicular weight after single or fractionated doses of X rays*

Expt. No.	Dose (*r*)					Mean weight (*mg.*)	
	Day of experiment					Day of experiment	
	0	1	2	3	4	27–29	30–32
1a*.....	0					228 (10)†	
	80					156 (5)	
	40		40			155 (5)	
	40				40	154 (6)	
1b.....	120					129 (6)	
	60		60			113 (6)	
	60				60	128 (6)	
1c.....	240					95 (6)	
	120		120			90 (5)	
	120				120	93 (5)	
2‡.....	0					230 (6)	
	100					137 (11)	149 (4)†
	60	40				131 (6)	
	60		40			126 (6)	135 (5)
	60			40		130 (6)	139 (6)
3§.....	100					136 (20)	
	20		30		50	133 (9)	138 (9)
	15	15	20	25	25	128 (10)	136 (10)
4§.....	0					238 (9)	
	100					126 (8)	135 (9)
	15	15	20	25	25	132 (7)	132 (9)

*Physically equipotent fractional doses.
†Number of mice in each group.
‡Non-equipotent fractional doses.
§Biologically equipotent fractional doses.

Results

The average weight of the testes of the control groups in table 1 was in the range 228 to 238 mg. Previous work (*9*) had shown that approximately 75 percent of the weight, or 170 mg., can be lost following irradiation. In most of the experiments the total dose, in the neighborhood of 100 r, was such that approximately 90 mg. or half the maximum weight loss occurred. Under these circumstances it was possible to demonstrate either an increased or decreased effect due to fractionation. The changes following a single dose of 100 r are shown in text-figure 1 (triangles). Approximately 1 week after exposure on *day 0* the weight began to fall, reaching a minimum on *day 27*, and rising again after about *day 32*.



TEXT-FIGURE 1.—Testicular weight following a dose of 100 r, given in a single dose (△) or in 5 fractions (○). The difference between the points at 21 days was statistically significant ($P \approx .02$) by the t test. The maximum losses, however, were within 6 percent of each other. These data are from experiment 4.

In an exploratory experiment comparing the effect of two fractions (40 r + 40 r and 80 r + 40 r) with that of a physically equivalent single exposure, weights were determined at 28 days after both the first and second fractions. Fractionation diminished weight loss when the fractions were separated by about 2 weeks, but not when separated by 1 week or less. Since an increase in damage due to fractionation was being sought, the main experiments were planned for 2 to 5 fractions in 1 to 4 days' elapsed time.

Three types of fractional doses were tested: physically equipotent (experiment 1), biologically equipotent (experiments 3 and 4), and those neither physically nor biologically equipotent (experiment 2).

Each fraction of the biologically equipotent doses caused equal amounts of weight loss and was planned on the basis of previous results (*9*). The weight in milligrams W at 28 days after a single radiation dose D of less than 150 r is adequately predicted by

$$W = 138\, e^{-0.011\, D} + 92.$$

The biologically equipotent doses of experiments 3 and 4 were calculated from this equation.

The results of the four experiments are shown in table 1. The testes were weighed at 27 to 29 days, and also in some experiments at 30 to 32 days, after the first exposure on *day 0*. The two weighings insured that the weight would be determined at the time of its minimum, even though fractionation delayed this event.

The data (table 1) show that fractionation of dosage did not change the maximum amount of weight lost. The rate at which the final effect was reached was somewhat slower under fractionated dosage (text-fig. 1). This was not surprising since the total dose was reached later than under nonfractionated dosage.

The dry weights of the testes, expressed as percentages of the fresh weights, increased slightly as the testes lost weight following irradiation, both in the single dose (SD) and the fractionated dose (FD) series of experiment 4.

Day:	*0*	*3*	*7*	*11*	*21*	*27*	*32*	*42*
SD:	14.8	14.9	14.7	14.9	15.1	16.2	15.4	14.3
FD:	14.8	15.4	14.7	14.8	15.4	16.2	15.8	15.4

The small but significant increase in dry weight was maximal when testicular weight was minimal, declining thereafter as normal weight was restored.

Discussion

It was shown previously (*9*) that the mouse testis reacts to X rays as if composed of two independent populations of biological units. Population *A*, controlling 60 percent of the total weight, is 25 times as sensitive to X rays as population *B*, and has an ED_{50} of 55 to 75 r. It was concluded that only one radiologic event is required to inactivate one *A* or *B* unit, since the weight loss in each population is an exponential function of radiation dose and is independent of the dose-rate (1.4 to 64 r/min.). The new fact that fractionation of dose over a 4-day period does not affect the results is further evidence in favor of the proposed single-action radiologic mechanism. The terms *radiologic event* and *single-action* are employed to avoid connotations that may be associated with the terms *hit* and *one-hit* of the so-called target theory. In the hamster and rat there is only one sensitive population, with an ED_{50} of about 110 r (*9*).

The spermatogonia are the most sensitive cells in the germinal epithelium of the rat and mouse and may be killed outright (*11–13*) or may give rise to progeny which die (*6*). It was therefore inferred that population *A* is composed of spermatogonia. Regaud and Blanc (*11*) distinguished "dusty" spermatogonia, now called type-A, from "crusty" spermatogonia, now called type-B, the latter being the more sensitive. Currently proposed schemes of spermatogenesis (*14–16*) suggest that a sequence of three type-A divisions followed by two type-B divisions in the course of 4 to 5 days leads to the production of the primary spermatocytes in rodents.

Which of the two types of spermatogonia corresponds to the radiologically defined units called population *A* depends upon which is the principal

factor limiting testicular weight under the conditions of the experiment. The importance of type-B is diminished by the fact that it follows type-A in spermatogenesis. For this and other reasons, it seems to us more likely that the type-A cell will prove to be the unit of population *A*. In either case, it should be noted that the exponential nature of the inactivation curve predicts the survival of a few spermatogonia at high doses—in the mouse 1 percent after 400 r and 0.01 percent after 800 r; in the hamster and rat 1 percent after 800 r and 0.01 percent after 1,600 r.

The conclusion that fractionation is without effect upon radiologic damage in the testes, when stated without qualification, is at variance with the literature.[4] Schinz and Slotopolsky (*1*), stimulated by Regaud's work on protraction of dosage (*5*), administered to the rabbit testes a total dose of 1 HED (skin erythema dose), equivalent to about 600 r (air), HVL = 1 mm. Cu. Injury was ascertained by histologic examination. When the dose was not fractionated, extensive though incomplete involution of the germinal epithelium occurred, followed by restitution 7 weeks after exposure. When the dose was given in 5 or 10 equal daily fractions, the epithelium consisted only of Sertoli cells at 7 weeks after exposure, and restitution occurred at 13 weeks. The authors emphasized that the primary effect of fractionation was delay of restitution. Their work is notable because it is the first demonstration that fractionation enhances the effect of X rays.

Sartory and Meyer (*2*) irradiated the testes of the rabbit, using the beam from a 200 kvc machine filtered with 2 mm. Cu + 1 mm. Al. Total doses of 750 to 3,750 r (skin ?) were given in a single exposure or in 10 to 12 equal fractions at 48-hour intervals. The data indicated that recovery was more likely following a single exposure than following the fractionated series.

Regaud and Ferroux (*17*), using 4 rabbits, concluded that fractionation spared the skin of the scrotum, although the effects upon the germinal epithelium were unchanged, or perhaps slightly increased. Subsequently, Ferroux, Regaud, and Samssonow (*3*) administered to the genital area of the rabbit a total dose of 2,040 r (air) in 2 to 6 daily fractions. The 180 kv. beam was filtered with 8 mm. Al and 15 mm. wood. The testes were examined histologically at 4 and 10 months after irradiation for the signs of permanent sterilization. It was concluded that sterilization was achieved only when the total dose was given in 4, 5, or 6 fractions, and that the Sertoli cells were better conserved under such dosage.

Fogg and Cowing (*18*), using 100 kvp X rays, HVL = 2.6 mm. Al, treated the pelvic region of C57BL mice with a total dose of 1,440 r (air) in one exposure and in 5 fractions at 4-day intervals. They examined the testes histologically up to 58 days after the first exposure. The necessity for making comparisons between such series at strictly comparable post-irradiation times had been discussed by Schinz and Slotopolsky (*1*), but Fogg and Cowing, who cited none of the references above (*1–3*, *17*), failed to do so. For this reason, the significance of their conclusion that

[4] In mice of the same strain there is approximately 70 percent recovery in a 4-day period from a fractional dose of 350 r total-body irradiation, judged by its contribution to the LD^{30}.

fractionation produces a greater effect on spermatogonia and spermatocytes is not clear.

The experiments cited thus far dealt with the effects of fractionation alone. Kirchhoff and Kelbling (*19*) studied the histologic effect in the rabbit testes of protracting fractionated doses. The term protraction is used specifically to indicate a decrease in dose-rate. Doses totaling 200 to 1,800 r were given in 1 to 10 daily fractions at either 73 or 2 r per minute. No differences due to dose-rate were observed at 5 or at 31 to 43 days after exposure. Lorenz (*20*) found no difference in testicular weight of the mouse at the end of treatment when 10 r per day were given for 30 days at 7 r per minute instead of 0.04 r per minute.

In contrast to the foregoing, when either protraction or fractionation was sufficiently extreme, the effect of a given total dose was diminished. In their studies in the tolerance range, Lorenz, Heston, Eschenbrenner, and Deringer (*7*) and Eschenbrenner, Miller, and Lorenz (*8*) showed that testicular weight in the mouse remained practically unchanged for 16 months of irradiation at 1.1 r per 8 hours per day. At 4.4 r per 8 hours per day, the weight fell to 60 percent of normal during the first 2 months, and in the next 14 months fell only 15 percent more.

Upon review, it is evident that several major technical differences distinguish the present series of experiments from previous ones. When fractionation decreased radiologic injury, extremely protracted fractionated dosage was employed, *e.g.* 0.01 r per minute for a total of 5 r per day administered for a period of months. When fractionation increased radiologic injury, the total dose was from 6 to 20 times greater than that investigated in the present experiments and the biologic effect measured was ability to recover from injury rather than injury *per se*. It is concluded that the present results complement rather than contradict the older ones.

When all the data pertaining to the effects of fractionation upon the testes are considered together, the outlines of some generalizations begin to emerge. Granting that more work needs to be done, the following synthesis is suggested. Extreme fractionation and protraction tend to diminish the degree of testicular involution by permitting the rate of biologic recovery to approximate that of radiologic injury. At dose-rates of 0.1 to 100 r per minute, the amount of involution for a given dose is independent of the dose-rate. The effect of fractionation then depends upon the size of the fractional dose, the interval of time elapsing between fractions, and the total dose. A total dose of about 100 r causes marked involution that is unchanged when the same total dose is administered fractionally in 1 to 4 days. Larger doses of 600 to 2,000 r cause maximum involution and also diminish the rate and final amount of restitution. Their fractional administration (daily, or 4 or 5 times weekly) throughout elapsed periods of 3 days to 3 weeks does not affect the degree of involution, but does increase the damage sustained by the mechanism responsible for restitution.

Summary

1) Following exposure to X rays, radiologic injury in the germinal epithelium of adult CAF_1 mice was measured by the maximum loss of testicular weight, which in the present experiments occurred at about 4 weeks after irradiation.

2) The effects of undivided and fractionated dosage were compared. The total dose was varied from 80 to 240 r (tissue). The number of fractions was varied from 2 to 5, given in 1 to 4 days' elapsed time. Physically equipotent, biologically equipotent, and non-equipotent fractions were used.

3) The degree of radiologic injury was proportional to the total dose, but it was not affected by fractionation within the limits used. This adds further support to the hypothesis advanced previously that one effective radiologic event inactivates one biologic unit, most likely a spermatogonium.

4) The present results, together with those in the literature cited, lead to the following synthesis which is proposed for the available data relating to the rodent testes. Extreme protraction and fractionation diminish injury. At dose-rates of about 0.1 to 100 r per minute the effect of fractionation is influenced by the size of the total dose. Doses of 100 r cause involution, but fractionation during a period of 4 days does not influence the amount. Doses of 600 to 2,000 r cause involution and also inhibit restitution. Fractionation of such large doses increases damage in the mechanism responsible for restitution.

References

(*1*) SCHINZ, H. R., and SLOTOPOLSKY, B.: Experimenteller Beitrag zur Frage der Röntgenallergie. Acta radiol. 7: 365–404, 1926.

(*2*) SARTORY, A., SARTORY, R., and MEYER, J.: Phénomènes apportés par l'irradiation sur le tissue cutané et sur la glande génitale mâle du lapin en fonction du mode d'application du rayonnement. Compt. rend. Acad. sc. 192: 447–449, 1931.

(*3*) FERROUX, R., REGAUD, C., and SAMSSONOW, N.: Comparison des effets produits sur les testicules du lapin, au point de vue de la stérilisation de l'épithélium séminal, par une même dose de rayons X, selon qu'elle a été administrée sans fractionnement ou bien fractionnée et étalée dans le temps. Compt. rend. Soc. biol. 128: 173–176, 1938.

(*4*) FRIEBEN: Hodenveränderungen bei Tieren nach Röntgenbestrahlung. München med. Wchnschr. 50: 2295, 1903.

(*5*) REGAUD, C.: Influence de la durée d'irradiation sur les effets déterminés dans le testicule par le radium. Compt. rend. Soc. biol. 86: 787–790, 1922.

(*6*) VON WATTENWYL, H., and JOËL, C. A.: Die Wirkung der Röntgenstrahlen auf den Rattenhoden. III. Verlauf der Degeneration bzw. Regeneration des Samenepithels nach Bestrahlung mit 60 bis 2400 r bis zu 50 Tagen nach der Bestrahlung. Strahlentherapie 70: 588–631, 1941.

(*7*) LORENZ, E., HESTON, W. E., ESCHENBRENNER, A. B., and DERINGER, M. K.: Biological studies in the tolerance range. Radiology 49: 274–285, 1947.

(*8*) ESCHENBRENNER, A. B., MILLER, E., and LORENZ, E.: Quantitative histologic analysis of the effect of chronic whole-body irradiation with gamma rays on the spermatogenic elements and the interstitial tissue of the testes of mice. J. Nat. Cancer Inst. 9: 133–147, 1948.

(9) KOHN, H. I., and KALLMAN, R. F.: Testes weight loss as a quantitative measure of x-ray injury in the mouse, hamster, and rat. Brit. J. Radiol. 12: 586–591, 1954.

(10) ESCHENBRENNER, A. B., and MILLER, E.: Effect of roentgen rays on the testis. Arch. Path. 50: 736–749, 1950.

(11) REGAUD, C., and BLANC, J.: Action des rayons X sur les diverses générations de la lignée spermatique. Extrême sensibilité des spermatogonies a ces rayons. Compt. rend. Soc. biol. 58: 163–165, 1906.

(12) BLANC, J.: Action des Rayons X sur le Testicule. These, Lyon, A. Storck et Cie, 1906, p. 73.

(13) OAKBERG, E. F.: Degeneration of spermatogonia of the mouse following exposure to x-rays and stages in the mitotic cycle at which cell death occurs. Personal communication.

(14) LEBLOND, C. P., and CLERMONT, Y.: Spermiogenesis of rat, mouse, hamster and guinea pig as revealed by the "periodic acid-fuchsin sulfurous acid" technique. Am. J. Anat. 90: 167–215, 1952.

(15) ROOSEN-RUNGE, E. C.: Kinetics of spermatogenesis in mammals. Ann. New York Acad. Sc. 55: 574–584, 1952.

(16) CLERMONT, Y., and LEBLOND, C. P.: Renewal of spermatogonia in the rat. Am. J. Anat. 93: 475–501, 1953.

(17) REGAUD, C., and FERROUX, R.: Discordance des effets des rayons X, d'une part dans la peau, d'autre part dans le testicule par le fractionnement de la dose: diminution de l'efficacité dans la peau, maintien de l'efficacité dans le testicule. Compt. rend. Soc. biol. 97: 431–434, 1927.

(18) FOGG, L. C., and COWING, R. F.: Effect of fractionated doses of radiation on normal and tumor tissue. Cancer Res. 13: 321–326, 1953.

(19) KIRCHHOFF, H., and KELBLING, W.: Experimenteller Beitrag zum Zeitfacktorproblem. (Histologische Studien am Kaninchenhoden zur Klärung des Einflusses der Protrahierung bei der Coutard-Bestrahlung). Strahlentherapie 60: 444–465, 1937.

(20) LORENZ, E.: Personal communication, 1952.

The Radiation Recovery Factor: Preservation by the Polge-Smith-Parkes Technique[1]

DAVID W. H. BARNES *and* JOHN F. LOUTIT,[2]
Medical Research Council's Radiobiological Research Unit, Atomic Energy Research Establishment, Harwell, Berks, England

Egon Lorenz and his collaborators in a series of papers (*1–5*) over the past few years made a number of notable advances in the treatment of experimental animals given lethal irradiation of the whole body.

Jacobson *et al.* (*6*) had shown that mice so irradiated could survive following implantation of normal splenic tissue into the peritoneal cavity. Lorenz *et al.* (*1*) showed that similar recovery was also obtainable with bone marrow, not only in mice but also in guinea pigs. Bone marrow, unlike spleen, is not a discrete organ, so Lorenz' method of administration of the tissue was by injection of a suspension. This was a considerable gain in technique. By intravenous, rather than intraperitoneal injection, smaller doses gave better survival—a great gain in therapeutic result. Furthermore, and most important, it was shown that guinea-pig marrow could cause a lethally irradiated mouse to survive (*2*). Jacobson (*7*) had postulated that the recovery factor in spleen (and bone marrow) was a humoral agent. By far the most potent argument in favor of this hypothesis, rather than one involving seeding of the donated cells in the recipient, was this demonstration that the recovery factor was not species specific. It is difficult to envisage how heterospecific cells could survive, multiply, and function. Further studies at Bethesda have resulted in reports that, in addition to guinea-pig marrow, rat bone-marrow (*4*) and bone-chips from mice and rats (*5*) are also active in the recipient mouse. These results and chemical evidence (*8, 9*) add further weight to the humoral theory.

On the other hand we have argued (*10, 11*) that much of the evidence is equivocal and that some fits better the hypothesis implicating a cellular graft from donor to recipient. Whatever the active agent, there is general agreement that it is probably macromolecular, particulate, and very labile *in vitro*. It loses its activity within a few hours at room temperature and is inactive, certainly after 48 hours at +4° C. and −15° C., and probably earlier. This is similar to the viability of mammalian cells.

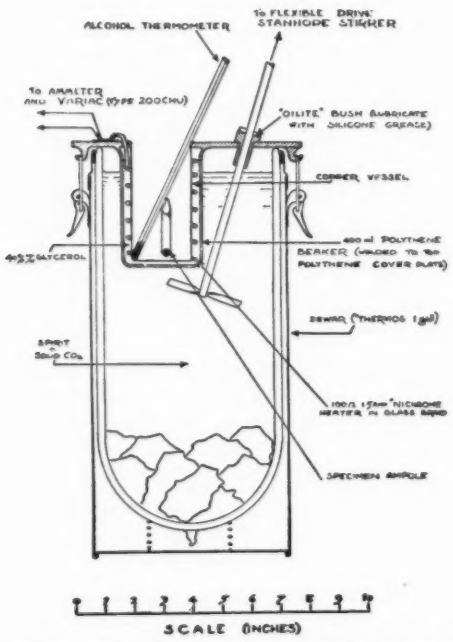
---

[1] Received for publication October 11, 1954.
[2] The authors are greatly indebted to Dr. A. S. Parkes and Dr. A. U. Smith for advice and to Miss D. K. Salmon for technical assistance.

However, Parkes and his collaborators (*12*, *13*) have demonstrated that the viability of many mammalian cells and tissues can be preserved by storage in glycerol at the temperatures of solid CO_2 freezing mixtures or of liquid air. We have therefore submitted infant mouse spleen to a scheme suggested by Parkes and Smith (personal communication) and have demonstrated that, thus preserved, it retains its ability to cause recovery of irradiated mice.

Apparatus

The apparatus (text-fig. 1) required for freezing the spleen was constructed in our laboratory workshop and is a small version of that used by Polge and Lovelock (*14*).


ALCOHOL THERMOMETER
To FLEXIBLE DRIVE STANHOPE STIRRER
To AMMETER AND VARIAC (TYPE 200CHU)
"OILITE" BUSH (LUBRICATE WITH SILICONE GREASE)
COPPER VESSEL
400 ml POLYTHENE BEAKER (WELDED TO 300 POLYTHENE COVER PLATE)
40% GLYCEROL
DEWAR (THERMOS 1 gal)
SPIRIT + SOLID CO2
100Ω 15w NICHROME HEATER IN GLASS BEAD
SPECIMEN AMPOLE
SCALE (INCHES)

TEXT-FIGURE 1.—Diagram to scale of apparatus used.

It consists of a one-gallon Dewar flask ("Thermos") containing freezing mixture of solid CO_2 in methylated spirit. Into the flask can be inserted a 300 ml. polythene beaker which has been welded to a sheet of polythene to act as a temporary cover. The polythene beaker contains 40 percent glycerol which freezes at −15° C., and has suspended in it a copper vessel. The copper vessel contains the sample tubes and a small quantity of spirit to prevent adhesion of the sample tubes at low temperature.

The theory behind the construction of the apparatus is that on placing the polythene beaker into the freezing mixture the temperature in the copper vessel falls slowly to −15° C. At this point the glycerol freezes and the temperature of the samples then falls rapidly to −70° C.

Our apparatus did not show the exact physical characteristics required; it was thus necessary to insert a heating coil in the 40-percent glycerol contained in the polythene beaker. The amount of heat applied is controlled by an ammeter and variable resistance.

Procedure

The spleens were excised from mice of strain CBA, 5 to 10 days of age, and chopped into fragments of about 1 $mm.^3$. These were placed in ampules containing 15 percent volume/volume glycerol in homologous serum. The glycerol for pipetting was taken from sterile ampules of 75 percent glycerol (A. R.) in physiological saline, since pure glycerol is too viscous for accurate pipetting.

The sealed ampules containing the chopped spleens in glycerol serum were placed in the copper vessel suspended in the polythene beaker. The whole assembly was then transferred to the flask of freezing mixture. The rate of fall of temperature of the samples was carefully followed with an alcohol thermometer and stop watch, and controlled by altering the current passing through the heating coil. The temperature fall was limited to 1° C. per minute down to −15° C. It was then allowed to fall to −70° C. at not more than 10° per minute. When the temperature of the ampules reached −70° C., they were suspended from corks and placed directly in the freezing mixture for prolonged storage. The polythene beaker assembly was removed and the normal insulated lid of the Dewar flask was replaced.

The flask was refilled with solid CO_2 as required. Losses were minimized over weekends by placing the flask in a refrigerator at −15° C.

When the spleens were to be injected into irradiated animals, the ampule required was removed from the freezing mixture and thawed rapidly in a water bath at 37° C. After opening the ampule, the glycerol serum containing the lumps of spleen was transferred to the vessel for homogenization.

Experimental Details

The mice for irradiation were males between 3 and 5 months old of the inbred CBA strain with which our previous studies were made. As before, five mice at a time were irradiated from below in the aluminum mouse box in which they live. The radiation was produced by a Westinghouse radiotherapeutic machine operated at 240 kv. and 15 Ma. and had a half-value layer equivalent to 1.2 mm. Cu with the uniformity filter in use. The dose delivered was equivalent to 950 r measured in air with backscatter at the position of the mice, and has been 100 percent lethal to over 300 control mice.

The irradiated mice were injected intravenously within an hour or so of irradiation.

In each experimental batch they consisted of three groups: 1) control mice who received only a dummy injection of sterile saline; 2) mice who received mush from spleens freshly excised; 3) mice who received freshly prepared mush from spleens stored at −70° C.

Results and Discussion

The results are summarized in table 1. They demonstrate that, as in our previous studies, the dose of 950 r is uniformly lethal in the absence of protective measures. The *positive* controls given mush from freshly excised and prepared spleens showed some variability but on the whole clinically satisfactory results—23 survivors out of 29 given the equivalent of one spleen and 14 out of 25 given the equivalent of ⅕ of a spleen. The mice given spleen stored for various lengths of time—2, 9, 17 days—also showed variable survival. There are sufficient survivors to demonstrate qualitatively that the activity of the preparation can be preserved for some weeks—19 survivors out of 30 given the equivalent of one spleen and 8 survivors out of 26 given the equivalent of ⅕ of a spleen. It is doubtful if it is profitable in such a small and preliminary experiment to discuss quantitative aspects. Inspection of the table suggests that the variance within groups of treated mice is as great as the variance between groups. Although the effect of injection of glycerol serum alone has not been adequately tested, in limited trials to date, no recovery has been scored.

TABLE 1.—*Thirty-day survival of strain CBA mice after 950 r*

Group	Injected intravenously with	Amount injected—equivalent of infant spleen	Number surviving/number mice						
			Lots						Total
I (control).	Sterile saline....	---	0/5	0/5	---	0/10	0/10	0/10	0/40
II.....	*Spleen mush*								
	fresh.....	1	4/4	4/5	5/5	5/5	0/5	5/5	23/29
	fresh.....	1/5	3/5	5/5	---	5/5	0/5	1/5	14/25
III.....	*Spleen mush stored at −70° C.*								
	for 2 days.....	1	4/5	1/5	0/5	---	5/5	---	10/20
	for 9 days.....	1	---	---	---	---	---	5/5	5/5
	for 17 days.....	1	---	---	---	4/5	---	---	4/5
	for 2 days.....	1/5	0/2	0/5	1/5	---	0/5	---	1/17
	for 9 days.....	1/5	---	---	---	---	---	3/4	3/4
	for 17 days.....	1/5	---	---	---	4/5	---	---	4/5

Another experiment with material stored for 83 days has been completed with the following results at 28 days after irradiation of mice with 950 r. The survivors were 0 out of 3 given saline; 5 out of 5 given the equivalent of 1 spleen fresh; 4 of 5 and 3 of 4 given the equivalents of 1 and ⅕, respectively, of spleen stored at −70° C.

Summary

Spleens of infant CBA mice, when injected intravenously as a mush, can lead to recovery of homologous adult mice given an otherwise lethal dose of X rays. The recovery factor has not been lost when the infant spleens have been stored in glycerol serum at −70° C. for as long as 83 days.

References

(*1*) Lorenz, E., Uphoff, D., Reid, T. R., and Shelton, E.: Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J. Nat. Cancer Inst. 12: 197–201, 1951.

(*2*) Lorenz, E., Congdon, C. C., and Uphoff, D.: Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. Radiology 58: 863–877, 1952.

(*3*) Congdon, C. C., Uphoff, D., and Lorenz, E.: Modification of acute irradiation injury in mice and guinea pigs by injection of bone marrow: a histopathologic study. J. Nat. Cancer Inst. 13: 73–107, 1952.

(*4*) Congdon, C. C., and Lorenz, E.: Humoral factor in irradiation protection: Modification of lethal irradiation injury in mice by injection of rat bone marrow. Am. J. Physiol. 176: 297–300, 1954.

(*5*) Lorenz, E., and Congdon, C. C.: Modification of lethal irradiation injury in mice by injection of homologous or heterologous bone. J. Nat. Cancer Inst. 14: 955–961, 1954.

(*6*) Jacobson, L. O., Simmons, E. L., Marks, E. K., Gaston, E. O., Robson, M. J., and Eldredge, J. H.: Further studies on recovery from radiation injury. J. Lab. & Clin. Med. 37: 683–697, 1951; correction 38: 330, 1951.

(*7*) Jacobson, L. O.: Evidence for a humoral factor (or factors) concerned in recovery from radiation injury: *A review.* Cancer Res. 12: 315–325, 1952.

(*8*) Cole, L. J., and Ellis, M. E.: Studies on the chemical nature of the radiation protection factor in mouse spleen. Radiation Res. 1: 347–357, 1954.

(*9*) Kelly, L. S., and Jones, H. B.: Influence of homologous tissue factors on DNA turnover and radiation protection. Am. J. Physiol. 172: 575–578, 1953.

(*10*) Barnes, D. W. H., and Loutit, J. F.: What is the recovery factor in spleen? Nucleonics 12: 68–71, 1954.

(*11*) ———: Spleen protection: the cellular hypothesis. Liege, Internat. Symp. Radiobiology, 1954. In press.

(*12*) Polge, C., Smith, A. U., and Parkes, A. S.: Revival of spermatozoa after vitrification and dehydration at low temperatures. Nature 164: 666, 1949.

(*13*) Smith, A. U.: The use of glycerol for preservation of living cells at low temperatures. Proc. Roy. Soc. Med. 47: 57–60, 1954.

(*14*) Polge, C., and Lovelock, J. E.: Preservation of bull semen at −79° C. Vet. Rec. 64: 396–397, 1952.

On Wasted Radiation and the Interpretation of Experiments With Chronic Irradiation[1,2]

R. H. MOLE,[3] *Medical Research Council's Radiobiological Research Unit, Atomic Research Establishment, Harwell, Berks, England*

There are perhaps two distinct aims in experimental work on irradiation effects. One is to understand the mechanism of radiation damage; the other is to discover how much radiation is required to do a given amount of damage. Here the most fundamental difficulty is the extrapolation from animal to man, a difficulty which increases inordinately with the duration of time over which the effects of radiation are sought. Lorenz (*1*) faced this problem when discussing the results of chronic irradiation of mice with *gamma* rays, and based his discussion of hematopoietic and ovarian damage on the idea that man should be considered as sensitive as is that species of animal found experimentally to be most sensitive to the particular form of radiation damage under consideration. In this paper this extrapolation difficulty will be put aside, and it will be assumed that the results of animal experiments with chronic irradiation are somehow transferable to man. The question to be examined is the quantitative relation between radiation dose and effect in such experiments.

In all the experiments reported until now (*2–7*), radiation has been given daily to a group of animals until all the animals were dead. The day of death of each animal gave the total radiation dose it accumulated, and the mean accumulated dose of a group has been the parameter used in discussing dose-effect relationships. One obvious question is, how much of the radiation was wasted? Supposing the irradiation had been stopped sometime before the death of an animal, would the animal have died just the same? Lorenz (*1*) was aware of this possibility that the mean accumulated dose was greater than the minimum dose required to produce the same effect, but considered that the difference between the two, or the wasted radiation, was likely to be relatively small. The experiment to be described shows, however, that (under some conditions at least) the wasted radiation may amount to well over half the mean accumulated dose. This in turn implies some revision of accepted dose-effect relationships.

[1] Received for publication October 18, 1954.

[2] This paper is based on a communication to the Seventh International Congress of Radiology, Copenhagen, Denmark, 1954.

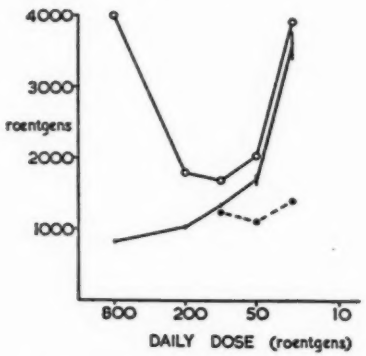
[3] The author is indebted to Mr. M. Corp, who carried out the irradiations, and to Mr. G. Jeffries for their help

Methods

Female strain CBA mice bred in the laboratory were used. Batches of 10 to 30 boxes of litter mates aged 8 to 11 weeks were randomly distributed so that each experimental cage held 5 mice. Food was a standard cube ration given *ad libitum*. The experimental treatment consisted of daily X irradiation at a dose of 200 r, 100 r, 50 r, 25 r, or 10 r daily. At each dose rate, certain total doses were decided on beforehand and allocated at random to the cages in a batch. The mice were irradiated 5 days a week until they had received their predetermined total dose. Irradiation then stopped and the cage was observed daily until all the mice in it were dead. A few cages were continuously irradiated 5 days a week at doses from 800 r to 25 r daily until all the occupants were dead. X rays were generated at 240 kv., 15 Ma., HVL, 1.2 mm. Cu giving a field uniform to ±5 percent with a stepped filter. The dose rate was 44 or 9.6 r per minute. The mice were irradiated in the ordinary aluminum cages in which they were maintained without being transferred to special exposure boxes. Each dead mouse was examined for the cause of death.

Results

The results of continued irradiation until all the mice were dead are given in text-figure 1 (*upper curve*) and show the same pattern of change with dose rate observed by others (*2, 8–10*). As the daily dose was decreased the mean accumulated dose decreased to a minimum and then rose. This should mean that as the dose rate decreased from 800 r daily to 100 r daily the killing efficiency of the radiation increased.



TEXT-FIGURE 1.—The relation between the daily dose of X rays (log scale) and the mean accumulated dose (*upper curve:* 0——0) and the LD_{50}—30 days (*lower curve:* ——) in strain CBA mice. The broken line (– – – –) represents the approximate course of the LD_{50} curve one year after the start of a limited exposure to daily irradiation.

A quite different relation between dose rate and the dose required to kill was given by those mice irradiated daily, not until they were all dead, but to a predetermined total dose (table 1). After such limited exposures

to daily doses of 200, 100, and 50 r, all the deaths occurring within the first few months after the irradiation occurred within 17 days of the end of the irradiation period. At 25 r daily, delayed deaths began 2 months after the end of the irradiation at total doses of 3,000 r and more, but this did not invalidate the determination of the LD_{50} at 30 days. The LD_{50} was determined by relating probit of deaths to the dose in roentgens. There was no internal evidence that the logarithm of the dose was a better parameter to use. In contrast to the results of continued irradiation there was a continuous increase in the LD_{50} as the daily dose was decreased from 800 r (text-fig. 1, *lower curve*). Thus, when wasted radiation was eliminated, there was a steadily decreasing killing efficiency of radiation as the daily dose was decreased. There was no minimum total dose for some intermediate dose rate.

TABLE 1.—*LD_{50}—30 days for fractional X irradiation of strain CBA mice*

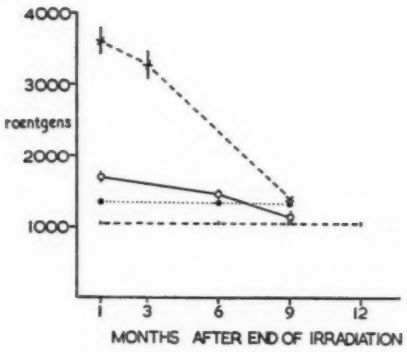
Daily dose rate	Mice used for determining LD_{50}	LD_{50}	S. E.	Reciprocal of probit-dose slope
r	*number*	*r*	*r*	*r*
200	40	1, 044	20	63
100	59	1, 344	31	154
50	75	1, 698	78	420
25	25	3, 600	200	620

The difference between the mean accumulated dose and the LD_{50} at 30 days was about the same, 320 to 340 roentgens at 100, 50 and 25 r daily. It might seem as if wasted irradiation became proportionately less as the daily dose was decreased, but the length of the period of observation after the end of irradiation is of crucial importance to any interpretation of these data. If mice survive 30 days after a single dose of radiation, few die in the next 12 months. This was also true of survivors of irradiation given at 200 r and 100 r daily. At 50 r and 25 r daily, however, delayed deaths occurred after the conventional 30 days, thus reducing the LD_{50} as the period of observation was prolonged (text-fig. 2). The LD_{50} at 25 r daily, 9 months after the end of irradiation, was less than half what it was 1 month after. These later deaths were undoubtedly due to the irradiation: control deaths were negligible in the same period. Similar though quantitatively different results have been obtained in male strain CBA mice, females of strain A, and the F_1 hybrid from crossing CBA and A.

Mice dying within 30 days of the end of the irradiation period commonly died with a severe anemia. Irradiated mice dying during the next year nearly always died of leukemia. The incidence of leukemia among controls during that time was nil. The tumor data will be reported in detail when the observation period has lasted longer.

Discussion

There have been very few experiments in which animals have been given daily doses of radiation of the order of twenty times the maximum per-



TEXT-FIGURE 2.—The change in LD_{50} after the end of a limited exposure of strain CBA mice to daily X irradiation.

|- - - - -| =200 r daily dose.
●. . . . .● =100 r daily dose.
○———○= 50 r daily dose.
×- - - -×= 25 r daily dose.

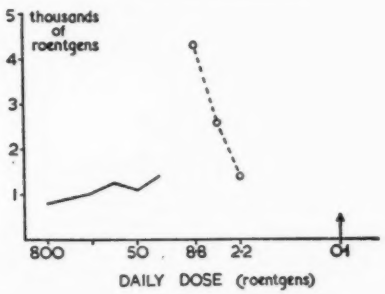
missible and less than this amount. The reasons are simple: the amount of work is very great since no measurable effect can be expected unless the irradiation is given over a large fraction of the animal's life span, the precautions required to insure that the control nonirradiated animals are treated in exactly the same way as the irradiated animals are onerous, and there is the ever-present danger that all the work will go for nothing because of some accident or epidemic. It is not surprising, therefore, that several attempts have been made to derive some relation between dose and effect, which will be true not only for single large doses of radiation and for repeated but still fairly large irradiations but which also can be extrapolated to discover the effects of dose rates of the order of the maximum permissible (*9*, *11–13*). Biologically, we may wonder if it is sensible to expect some single relation between radiation dose and effect over a 10,000-fold range of dose rate, but if some empirical relation did exist it would be very useful.

These attempts to extrapolate dose-effect relationships were all based on experiments involving continued irradiation until death. That this procedure may exaggerate the amount of radiation required to kill is obvious if one considers daily doses of the order of 1,000 r. One dose is enough to kill but the animal takes several days to die. On each of those days another dose can be given but all this subsequent radiation is wasted as far as the fact of death is concerned. Its only action can be to make death occur earlier and, interestingly, even this effect is not at all marked.

In experiments on mice and guinea pigs with *gamma* radiation at 90 to 140 r per day, Thomson, Tourtellotte, Carttar, Cox and Wilson (*10*) found that the LD_{50} for limited exposures was 40 to 65 percent of the mean accumulated dose for continued exposure. The experiment reported

here shows that the mean accumulated dose for continued exposure includes a variable but important proportion of wasted radiation at dose rates down to 25 r daily (text-fig. 1). Also, the proportion of radiation which can be considered wasted increases as the observation period is prolonged, especially at 25 r daily. It should perhaps be emphasized that the main human risk is from an exposure to radiation for limited periods followed by a lifetime much more nearly free from exposure, a situation which was imitated in the work reported here.

In text-figure 3 are shown the LD_{50}'s obtained here a year after the start of irradiation, and the mean accumulated doses found by Lorenz *et al.* (*4*) for chronic *gamma* irradiation of mice by 8.8 to 2.2 r daily. If it is true that the killing dose of radiation reaches a maximum at a daily dose somewhere between 25 and 4.4 r (as text-fig. 3 superficially suggests), then at smaller daily doses than this the killing efficiency of a roentgen must again increase. This effect might become very relevant to deciding what the maximum permissible level should be for a lifetime's exposure. On the other hand, if the mean accumulated doses found by Lorenz *et al.* (*4*) included an important proportion of wasted radiation, then it is probably safe to extrapolate and assume that the killing efficiency of a roentgen decreases steadily as the daily dose is decreased to maximum permissible levels. Although insufficient time has elapsed from the start of X irradiation at 10 r daily for a proper assessment, it seems probable that the LD_{50} at the end of a year will be much less than 4,000 r. With the reservation that the intensities at which daily irradiations are given may prove to be of critical importance, it seems safe to conclude that the mean accumulated dose of 4,300 r at 8.8 r daily [Lorenz *et al.* (*4*)] did include a considerable amount of wasted radiation, and that there is, so far, no evidence against the assumption that the killing efficiency of radiation decreases steadily as the daily dose is decreased.



TEXT-FIGURE 3.—A comparison of the results of continued *gamma* irradiation (Lorenz *et al.* (*4*), ○ — — — — ○) and limited X irradiation (from text-fig. 1).

There is a definite limit to the daily dose rates worth examining experimentally. As long as the period of observation is less than the time at which there is an appreciable death rate in the controls, no correction for control deaths is required. If the daily dose is low enough it will take

longer than this to accumulate enough radiation to have a detectable effect. The measurement of the LD_{50} when this includes an appreciable correction for natural mortality is inevitably suspect, though Sampford (*14*) has shown how one correction method may be applied to the data of Lorenz *et al.* (*4*) on chronic *gamma* irradiation. At 2.2 r daily the correction was large and when this is so the mean accumulated dose is, so to say, artificially diminished (text-fig. 3). If the natural life span had been longer some of the irradiated animals would have lived longer and therefore received more radiation. The shape of the control mortality curve for our strain CBA mice (*15*) is unfortunately not adapted to the use of this method, although eminently suitable for the sort of experiment under consideration because control mortality is negligible until after the mice are 1½ years old.

The experimental data also provide information on the rate of recovery after whole-body irradiation. Using paired doses it has been supposed that recovery takes place exponentially and at the rate of about 10 percent per day [for rats, Hagen and Simmons (*8*); for mice, Paterson, Gilbert and Matthews (*16*)]. At this rate our CBA mice could never be killed by 50 r daily on 5 days a week, let alone 25 r. If exponential recovery is still assumed for prolonged irradiation, then from the LD_{50} for deaths within 30 days it may be deduced that the over-all daily recovery rate cannot be more than 5 percent after 50 r daily and 2½ percent after 25 r daily (to be published). The conclusion is inescapable that continued irradiation damages the ability to recover.

This discussion, however, ignores the more important point that the delayed deaths which occur after a limited exposure to daily irradiation show that such irradiation has irreversible effects. A dose of 1,000 r, given at 50 r daily, killed about half the mice during the next year. This does not agree with Blair's analysis (*12*) from which he concluded that the average life expectancy was reduced by only 7 percent for each 1,000 r of the total accumulated dose. Blair assumed that the recovery rate was constant and not affected by previous doses of radiation which, as has been seen, is in conflict with the facts. The data used in his analysis were derived from experiments where animals were irradiated continuously until death and, as already pointed out, mean accumulated dose is not in fact a suitable parameter to use. Nor is mean survival time, the other parameter, since this depends not so much on the dose rate or total dose of radiation as on how long the biological response to the radiation takes to develop. There must always be some doubt of the real value of mathematical treatment of survival data where the kinds of biological failure that cause death are disregarded; but if one is trying to relate amount of radiation and effect it seems necessary to design experiments to avoid excess or wastage of radiation.

The rate of development of lethal lesions seems to be affected in a complex way by fractionation, as Kaplan and Brown (*17*) have already observed under different conditions for lymphoid-tumor development in C57BL mice. After 1,000 r given in one dose to mature female strain

CBA mice death takes a few days and after the same total dose, given at 100 r daily, deaths were delayed for over a year (since leukemia occurred rarely), but when the mice were given 50 r daily the delay was shorter and the frequency of deaths greater. The efficiency of production of leukemia in CBA mice by X radiation appeared to increase as the daily dose of radiation was decreased, at least over the range of dose rates so far examined.

The relevance of mouse leukemia to the human disease is debatable (*1*, *18*, *19*), and it is legitimate to argue that, because most of the delayed deaths after a limited exposure of mice to daily irradiation were due to leukemia, man would not in fact have shown the same sort of delayed mortality. However different pathologically the two diseases may be, mouse and human leukemia show similarities in their causation by radiation. Both are increased in incidence by single doses of radiation [Furth and Upton (*20*)] and by chronic irradiation [March (*21*), Lorenz *et al.* (*4*), and this experiment]. Lorenz (*1*) suggested that the dose needed to increase the incidence of leukemia in mice was far greater than that received by radiologists, but Mitchell (*22*) suggested that physicians working with radiation used to receive doses of the order of 10 r daily, a dose rate which is sufficient to produce leukemia in CBA mice after less than 6 months' exposure.

Summary

The effects of continued daily irradiation until death and of a limited exposure to daily irradiation are contrasted. The killing efficiency per roentgen of a limited exposure is much greater than for continued irradiation because so much of the continued irradiation is wasted. When allowance is made for wasted radiation, the pattern of dose and effect for chronic irradiation is markedly altered. The irreversible consequences of a limited exposure are quantitatively important.

References

(*1*) Lorenz, E.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. VI. Conclusions and applicability of results to the problem of human protection. *In* Biological Effects of External X and Gamma Radiation, Part I. (Zirkle, R. E., ed.), NNES IV–22B, New York, McGraw-Hill, 1954, pp. 226–242.

(*2*) Henshaw, P. S.: Experimental roentgen injury. IV. Effects of repeated small doses of X-rays on blood picture, tissue morphology, and life span in mice. J. Nat. Cancer Inst. 4: 513–522, 1944.

(*3*) Boche, R. D.: Observations on populations of animals exposed to chronic roentgen irradiation. MDDC–204, 1946.

(*4*) Lorenz, E., Jacobson, L. O., Heston, W. E., Shimkin, M., Eschenbrenner, A. B., Deringer, M. K., Doniger, J., and Schweisthal, R.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. III. Effects on life span, weight, blood picture, and carcinogenesis and the role of the intensity of radiation. *In* Biological Effects of External X and Gamma Radiation, Part I. (Zirkle, R. E., ed.), NNES IV–22B, New York, McGraw-Hill, 1954, pp. 24–148.

(*5*) Henshaw, P. S., Snider, R. S., Riley, E. F., Stapleton, G. E., and Zirkle, R. E.: Comparative late effects of periodic doses of fission neutrons and of gamma rays. MDDC–1253, 1946.

(6) Evans, T. C.: Effects of small daily doses of fast neutrons on mice. Radiology 50: 811–834, 1948.

(7) Neary, G. J., Munson, R. J., and Mole, R. H.: Chronic irradiation of mice by fast neutrons. Unpublished data.

(8) Hagen, C. W., Jr., and Simmons, E. L.: Effects of total-body X-irradiation on rats. I. Lethal action of single, paired, and periodic doses. CH–3815 (Argonne National Laboratory), 1947.

(9) Sacher, G. A.: Survival of mice under duration-of-life exposure to X rays at various dose rates. CH–3900 (Argonne National Laboratory), 1950.

(10) Thomson, J. F., Tourtellotte, W. W., Carttar, M. S., Cox, R. S., Jr., and Wilson, J. E.: Studies of the effects of continuous exposure of animals to gamma radiation from cobalt 60 plane sources. Am. J. Roentgenol. 69: 830–838, 1953.

(11) Brues, A. M., and Sacher, G. A.: Analysis of mammalian radiation injury and lethality. *In* Symposium on Radiobiology. The Basic Aspects of Radiation Effects on Living Systems (Nickson, J. J., ed.). New York, Wiley, 1952, pp. 441–465.

(12) Blair, H. A.: A formulation of the injury, life span, dose relations for ionizing radiation. II. Application to the guinea pig, rat and dog. UR 207 (Univ. of Rochester), 1952.

(13) Mole, R. H.: The effect of chronic irradiation on life span. Unpublished data, cited by Loutit, J. F. Proc. Roy. Soc. Med. 43: 857, 1950.

(14) Sampford, M. R.: The estimation of response–time distributions. I. Fundamental concepts and general methods. II. Multi-stimulus distributions. Biometrics 8: 13–32; 307–369, 1952.

(15) Barnes, D. W. H., and Loutit, J. F.: Spleen protection: the cellular hypothesis. *In* International Symposium of Radiobiology (Alexander, P., and Bagg, Z. M., eds.). London. In press.

(16) Paterson, E., Gilbert, C. W., and Matthews, J.: Time intensity factors and whole body irradiation. Brit. J. Radiol. 25: 427–433, 1952.

(17) Kaplan, H. S., and Brown, M. B.: A quantitative dose-response study of lymphoid-tumor development in irradiated C57 black mice. J. Nat. Cancer Inst. 13: 185–208, 1952.

(18) Furth, J., and Lorenz, E.: Carcinogenesis by ionizing radiations. *In* Radiation Biology, Volume I (Hollaender, A., ed.). New York, 1954.

(19) Israels, M. C. G.: The shortcomings of animal research in leukaemia. *In* Leukaemia Research (Wolstenholme, G. E. W., and Cameron, M. P., eds.). London, 1954.

(20) Furth, J., and Upton, A. C.: Induction of leukaemia by ionizing irradiation. *In* Leukaemia Research (Wolstenholme, G. E. W., and Cameron, M. P., eds.). London, 1954.

(21) March, H. C.: Leukemia in radiologists in a 20-year period. Am. J. M. Sc 220: 282–286, 1950.

(22) Mitchell, J. S.: Personal communication, 1948.

Some New Experiments on Protection Against Whole-Body Irradiation[1, 2]

A. Lacassagne, J-F. Duplan, *and* N. P. Buu-Hoi, *Laboratoire Pasteur de l'Institut du Radium, Paris, France*

The general problem of protection against the biological action of radiation remains fundamentally a problem of the cell, whether irradiation is applied: *a*) to a microorganism, *b*) to a single organ, the rest of the body being protected by a screen, *c*) to most of the body of an animal, with the exception of some particular segment, or *d*) to the entire body.

In any case, the aim of these experiments is either 1) to prevent the death of cells by inhibiting certain lethal cytochemical reactions (primary or secondary), or 2) to promote the recovery of those tissues that have suffered reversible injury.

Unfortunately, the increasing complexity of associated reactions makes the interpretation of phenomena more and more difficult in the case of more highly evolved organisms that have been irradiated over a large surface. With laboratory animals, apart from chemical agents for the preservation and restoration of cells, the following biological substitution treatments have been used successfully: transfusion, parabiosis, segmentary protection, injection of living cells, tissue extracts or hormones.

The present report deals with three series of experiments chosen from those being carried out at the Institut du Radium in Paris. The first series concerns the preventive use of certain phenolic compounds on the mouse irradiated *in toto*; the second series concerns the use of glucosamine under the same conditions, and the third series describes the effect of embryonic rat-liver suspensions injected into rats after irradiation.

Tests for Protection with Phenolic Compounds

The use of phenolic compounds *in vivo* prior to irradiation was suggested by the results obtained by Loiseleur (*1*) who irradiated *in vitro* some of these substances, which react specifically with peroxidases, and also by the work of Fox (*2*) on the inhibition by certain phenols of the depolymerization of polystyrolene in a chloroform medium.

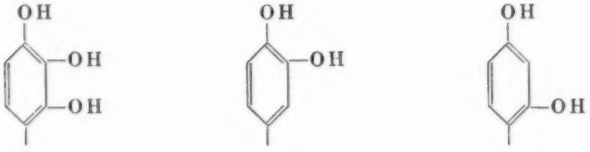
[1] Received for publication October 18, 1954.
[2] With the support of the Commissariat à l'Energie atomique.

Experiments on Mice Irradiated in Toto

a) Technique.—The mice, both male and female, belonging to strain XVII, were 60 to 90 days old and weighed from 20 to 25 gm. at the time of the experiments. At varying times before exposure to X rays, the mice each received a single intraperitoneal injection of 0.5 ml. of a solution of the compound under study, in neutralized olive oil. In certain cases, such as those of pyrogallol, resorcinol, and pyrocatechol, the solvent was physiological saline. It was shown that an injection of this solvent, or of a sodium salt of a fatty acid (Na laurate), had no effect on the outcome of irradiation.

X rays were given under the following conditions: 180 kv., 10 Ma., 0.3 mm. Cu, 65 r per minute. The dose, measured in air by means of a Mekapion, at a distance corresponding to the central region of the animals, was 700 r (100% lethal in 12 days for strain XVII). For the purpose of irradiation the mice were enclosed, six at a time, in a specially designed plastic box. The noninjected controls were treated in exactly the same manner.

b) Effects of polyphenolic compounds.—4-Acyls derived from pyrogallol (I) (*3*), pyrocatechol (II) and resorcinol (III) were used.



(I) (II) (III)

The results are given in table 1. One notices that the three polyphenols are inactive, but the acyls derived from pyrogallol have a definite pro-

TABLE 1.—*Tests for protective action of polyphenolic compounds against irradiation*

Substances injected	Dose (*mg./kg.*)	Delay between injection and X irradiation (*minutes*)	Survival rate on the 30th day*	Date of death
Controls	.....	.....	0/12	6–13
Pyrogallol	165	15	0/6	5–10
4-Caproyl-pyrogallol R=C_5H_{11}	300	30	3/9	6–11
4-Decanoyl-pyrogallol R=C_9H_{19}	400	60	5/12	11–23
4-Lauroyl-pyrogallol R=$C_{11}H_{23}$	450	30	4/10	11–24
4-Behenoyl-pyrogallol R=$C_{21}H_{43}$	750	45	4/10	11–21
Resorcinol	120	10	0/6	6– 9
4-Palmitoyl-resorcinol R=$C_{15}H_{31}$	400	30	0/6	7–12
Pyrocatechol	160	10	0/6	6–10
4-Behenoyl-pyrocatechol R=$C_{21}H_{43}$	600	45	1/10	8–16
	750	30	0/12	6–13

*Number surviving / number mice.

tective action when the lateral chain is a fatty acid with six or more carbons (C_6 to C_{22}). 4-Behenoyl-pyrocatechol seems to have a slight protective action (table 2), which action we were unable to find in the case of 4-palmitoyl-resorcinol.

TABLE 2.—*Effective time interval between injection of 4-behenoyl-pyrogallol and X irradiation.*

Time between injection and X irradiation (*minutes*)	Survival rate on the 30th day*
10	0/9
30	2/10
45	4/10
60	1/6
90	0/6
150	0/6

*Number surviving / number mice.

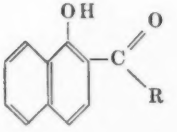
c) Duration of the protective effect.—To study this problem, 4-behenoyl-pyrogallol was used, with a uniform dose of 750 mg. per kg. (It is known that higher doses, injected 1 hour before irradiation, are toxic.)

Results with other compounds have confirmed those obtained by the use of 4-behenoyl-pyrogallol.

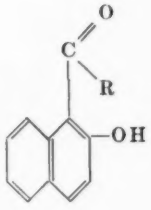
To conclude, it has not been possible, even with compounds with very long lateral chains, to obtain a protective effect lasting more than one hour.

Experiments With Naphthol Derivatives

Two acylated derivatives have been studied, 2-acyl-1-naphthol (I) and 1-acyl-2-naphthol (II)



I



II

It is known that the OH of naphthol is more reactive than those of phenols or polyphenols. Fox (*2*) considers naphthols, 1-naphthol as well as 2-naphthol (*4*), as very active protectors *in vitro*.

The results obtained with these compounds, when used under the conditions mentioned above to prevent the death of irradiated mice, fit in well with the theoretical data (table 3).

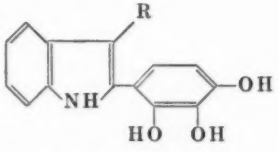
TABLE 3.—*Tests for protective action of naphthol derivatives against irradiation*

Substances injected	Dose (*mg./kg.*)	Time between injection and X rays (*minutes*)	Survival rate on the 30th day*	Date of death
1-Behenoyl-2-naphthol R=$C_{21}H_{43}$.....	250	35	6/10	10–20
2-Acetyl-1-naphthol R=C_2H_5.....	60	35	1/12	10–15
2-Palmitoyl-1-naphthol R=$C_{15}H_{31}$.....	160	30	0/6	7–12
2-Stearoyl-1-naphthol R=$C_{17}H_{35}$.....	200	30	1/6	8–15
2-Behenoyl-1-naphthol R=$C_{21}H_{43}$.....	200	30	2/6	8–18

*Number surviving / number mice.

Experiments With Other Complex Phenolic Derivatives

It was hoped to strengthen the protective action of polyphenols by fixing onto their molecule some other substance endowed with a protecting ability, like indole (*5*, *6*). Thus, a series of derivatives of the trihydroxyphenyl indole type were prepared.



3-R 2-(2'-3'-4'-trihydroxyphenyl) indole

No compound of this type showed any protecting activity. It has been impossible to ascertain whether these negative results were due to the fixation of the lateral chain onto the indole ring, or to the absence of a ketonic group on the polyphenolic ring.

Experiments on Protection by Glucosides

Baclesse and Loiseleur (*7*, *8*) have shown, some years ago, that induced hyperglycemia in rabbits delays the loss of hair from their areas of irradiated skin and causes regrowth of the hair to begin sooner. Furthermore, Loiseleur and Velley (*9*) noted that a high dose of glucose administered intraperitoneally to the rat has a radioprotective effect, and also that a mixture of cysteine, glucose and ascorbic acid, injected immediately after irradiation still affords a certain protective effect (*10*).

As it has been mentioned that the presence of an amine group reinforces the radioprotective action of certain chemicals that have previously shown protective activity (*11–21*), one of us, in collaboration with Rosenberg (*13*) has tested the action of glucosamine. The experimental conditions were the same as those described above. The results, summarized in table 4, show that glucosamine possesses important protective properties against the lethal effects of radiation.

TABLE 4.—*Test for protective action of glucosamine against irradiation*

Substances	Dose (*mg./kg.*)	Time between injection and irradiation (*minutes*)	Dose (*r*)	Survival rate on the 30th day*
Controls			580	0/6
Glucosamine	250	5	580	6/6
Controls			700	0/10
Glucosamine	300	5	700	8/16
Glucosamine	425	20	700	0/6

*Number surviving / number mice.

Neither glucose, fructose, hexose diphosphate, nor glutamic acid has shown the same activity as glucosamine when injected at a dose of 300 mg. per kg. under the same experimental conditions.

The fact that glucosamine, injected 20 minutes before irradiation, loses its protective action, leads us to think that this chemical is active by itself and not by means of its metabolites.

Experiments on Biological Substitution With Embryonic-Liver Cells

We owe to Lorenz and his co-workers (*14–16*) the knowledge that the intravenous injection of bone-marrow cells, immediately after whole-body irradiation of mice and guinea pigs, lowers the death rate and favors restoration of hemopoietic tissues.

On the other hand, workers from Maisin's laboratory (*17–20*) have insisted on the importance of liver in the mechanism of radioprotection,[3] as this organ, more than any other, when protected by a lead screen during a whole-body irradiation, provides the largest survival time for the rats submitted to a lethal dose of X rays.

Consequently one may think that the myeloid and hepatic factors that stimulate repair are both present at the same time in hemopoietic fetal liver.

Experiments With Rats Receiving Whole-Body Irradiation

Male Wistar-strain rats, weighing 200 gm., have received 725 r measured in air with a Mekapion, at a point corresponding to the center of the body. The irradiation conditions were 180 kv., 10 Ma., 0.3 mm. Cu, 70 cm.

After a delay varying between 4 and 18 hours, these animals received 0.5 ml. of a suspension of liver cells from fetal rats. The injections were given either intravenously or intraperitoneally.

To prepare the cell suspension, the fetuses were taken aseptically from the uterus between the 13th and 21st day of gestation. The livers were ground in physiological saline buffered at *p*H 7.2, either in a Potter homogenizer with a piston made of plastic instead of the ordinary glass piston or in a small Erlenmeyer flask containing glass beads (*21*). The

[3] In their latest paper (*20*) these authors have reversed their former stand and no longer admit that liver plays an essential part.

suspension was then filtered through cotton or gauze and the state of the cells was observed with a phase-contrast microscope. The final dilution was such that 1 ml. contained approximately 6×10^6 cells. All manipulations were carried out aseptically at a temperature of 4° C.

It can be seen, from the results given in table 5, that the cell suspension given intravenously is much more active than that given intraperitoneally. On the other hand, the activity of embryo livers seems to correspond to the degree of development of the hemopoietic function, as seen on smears of the livers used to prepare the suspension. It has not been determined which type of blood cell must predominate in the fetal livers in order to obtain the most active suspension.

TABLE 5.—*Protective action of fetal liver cells against irradiation of rats*

Injected with	Mode of introduction	Number of rats injected	Number of surviving rats
Physiological saline (controls)	intravenously	15	1
Cell suspension of			
13–14 day (whole fetus)	"	6	0
Of livers from 16-day fetus	"	6	1
Of livers from 20-day fetus	"	6	4
Of livers from 20-day fetus	intraperitoneally	6	0

It would be interesting to see whether the livers from younger embryos give a better therapeutic result when greater numbers of cells are injected. Unfortunately, these types of experiments are limited either by the production of embolism when the suspensions grow too rich in cells, or by the overly large quantity of liquid that has to be injected if the concentration of 6×10^6 cells per ml. is to be maintained.

Summary

The results of experiments on mice and rats receiving whole-body lethal doses of X rays are given.

The protective effect of certain chemicals has been studied. An effective diminution of mortality and a prolonged survival time have been obtained with phenolic and naphtholic derivatives containing long chain fatty acids. If the injection takes place more than one hour before irradiation these chemicals afford no protection. Glucosamine administered a few minutes before irradiation also provides some degree of protection for the animals.

Survival of rats receiving whole-body irradiation is increased by post-irradiation intravenous injection of an emulsion of liver cells from fetal rats.

Bibliography

(*1*) LOISELEUR, J.: Mise en évidence des états successifs d'oxydo-réduction dans les milieux soumis à l'action des rayons X. Bull. Soc. Chim. Biol. 25: 22–30, 1943.

(*2*) FOX, M.: Action de certains composés protecteurs sur la dégradation des polymères par les rayons X. Compt. rend. Acad. Sc. 237: 1682–1683, 1953.

(3) LACASSAGNE A., DUPLAN, J-F., and BUU-HOÏ, N. P.: Préservation contre les effets létaux des rayons X par les 4-acylpyrogallols chez la Souris. Compt. rend. Acad. Sc. 238: 1279–1281, 1954.

(4) FOX, M.: Personal communication.

(5) ALEXANDER, P.: A physico-chemical method of testing the protective action of chemical compounds against the lethal effects of ionizing radiations. Brit. J. Radiol. 26: 413–416, 1953.

(6) ALEXANDER, P., and FOX, M.: Polymérisation et dépolymérisation par les rayons X. Effet des agents protecteurs. J. Chim. Physique 50: 415–422, 1953.

(7) BACLESSE, F., and LOISELEUR, J.: Modifications de la radiosensibilité cutanée du lapin au cours de l'hyperglycémie expérimentale. Compt. rend. Soc. biol. 141: 743–745, 1947.

(8) LOISELEUR, J., and BACLESSE, F.: Sur la diminution de la radiosensibilité cutanée du lapin hyperglycémié. Compt. rend. Soc. biol. 141: 1160–1161, 1947.

(9) LOISELEUR, J., and VELLEY, G.: Immunité conférée par l'hyperglycémie contre les rayons X administrés à dose léthale. Compt. rend. Acad. Sc. 231: 182–184, 1950.

(10) ———: Traitement curatif des radiolésions consécutives à l'administration d'une dose léthale de Rayons X. Compt. rend. Acad. Sc. 231: 529–531, 1950.

(11) PATT, H. M., TYREE, E. B., STRAUBE, R. L., and SMITH, D. E.: Cysteine protection against X irradiation. Science 110: 213–214, 1949.

(12) BACQ, Z. M., HERVE, A., LECOMTE, J., FISHER, P., BLAVIER, J., DECHAMPS, G., LE BIHAN, H., RAYET, P.: Protection contre les rayons X par la B mercaptoethylamine. Arch. Int. Physiol. 59: 442–447, 1951.

(13) DUPLAN, J-F., and ROSENBERG, A. J.: Action préservatriee de la gluco-samine vis à vis de l'action létale d'une irradiation totale par rayons X. Chez la souris. Compt. rend. Acad. Sc. 239: 116–117, 1954.

(14) LORENZ, E., UPHOFF, D., REID, T. R., and SHELTON, E.: Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J. Nat. Cancer Inst. 12: 197–201, 1951.

(15) LORENZ, E., CONGDON, C. C., and UPHOFF, D.: Modification of acute irradiation injury in mice and guinea pigs by bone marrow injections. Radiology 58: 863–877, 1952.

(16) CONGDON, C. C., UPHOFF, D., and LORENZ, E.: Modification of acute irradiation injury in mice and guinea pigs by injection of bone marrow: A histopathologic study. J. Nat. Cancer Inst. 13: 73–107, 1952.

(17) MANDART, M., LAMBERT, G., MAISIN, H., and MAISIN, J.: Importance de la protection hépatique chez les rats soumis à une dose mortelle de rayons X. Compt. rend. Soc. biol. 146: 1647–1649, 1952.

(18) MAISIN, J., DUNJIC, A., VAN LANCKER, J., LAMBERT, G., and PASSEAU, L.: A propos de l'influence de la protection hépatique sur la survie des animaux irradiés in toto. Importance de la zone protégée. Compt. rend Soc. biol. 147: 1520–1522, 1953.

(19) VAN LANCKER, J.: De l'influence de la protection hépatique sur le comportement du système hemopoiétique des animaux irradiés in toto. Compt. rend. Soc. biol. 147: 1515–1517, 1953.

(20) MAISIN, J. H., DUNJIC, A., and MAISIN, H. E.: Importance de la protection de la région hépatique chez les rats soumis à une dose mortelle de rayons X. Compt. rend. Soc. biol. 148: 611–615, 1954.

(21) KAMINSKI, E. J.: A method for the preparation of isolated cells for use in high-resolution autoradiographic studies. Brit. J. Radiol. 26: 378–380, 1953.

Results of Exposure of Newborn Strain HR Mice to X Radiation [1,2]

MARGARET K. DERINGER *and* EGON LORENZ,[3]
National Cancer Institute,[4] Bethesda, Md.

Papillomas of the skin have been observed in a small number of untreated haired (*Hr/hr*) and hairless (*hr/hr*) animals of the HR strain and in 100 percent of such animals following painting with 20-methylcholanthrene (*1*). The present investigation was undertaken to ascertain whether the incidence of papillomas of the skin could also be increased with exposure to X radiation.

Materials and Methods

The HR stock of mice was obtained from Dr. F. G. Carnochan of Carworth Farms in 1947. The animals have been inbred by brother × sister matings since their arrival in this laboratory. Animals of generations F_4 through F_7 were used in this experiment. The hairless females failed to give sufficient milk to rear their young so matings were made using haired (*Hr/hr*) females and hairless (*hr/hr*) males. The young were irradiated within 12 hours following birth (with the exception of 2 litters, one of which was irradiated within 24 hours and one within 48 hours following birth) with a total dose of 400 r to the whole body. The physical factors of irradiation were as follows: 186 KVP, 20 Ma., filtration with 0.25 mm. Cu and 0.55 mm. Al, distance from focus to middle of mouse 50 cm., rate 62.4 r per minute, total dose 400 r. A total of 112 males (55 haired and 57 hairless) and 122 females (57 haired and 65 hairless) were irradiated.

The animals were housed in wooden cages, 8 animals to a cage, during the early months of the experiment but later were transferred to plastic cages. They were constantly supplied with Derwood food pellets and tap water. The animals were observed at weekly intervals. When they became moribund or died, autopsies were performed except for 72 animals (12 haired and 21 hairless females, 14 haired and 25 hairless males) in which post-mortem autolysis had developed. These 72 animals were omitted

[1] Received for publication September 28, 1954.

[2] This manuscript was prepared, in part, while one of the authors (M. K. D.) was a Summer Investigator at the Roscoe B. Jackson Memorial Laboratory in Bar Harbor, Maine.

[3] Deceased February 12, 1954.

[4] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

from the data. The kidneys, testes, ovaries, uterus, and vagina were routinely taken for section. Sections were also made of any tumor or any unusual lesion observed at autopsy. The tissues were fixed in Fekete's modification of Tellyesniczky's fixative (70 percent ethyl alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part) and stained with hematoxylin and eosin.

Results and Discussion

The types of tumors which were found in irradiated strain HR mice are listed in table 1.[5] Fifty-three of the irradiated males (32 hairless and 41 haired) and 39 of the irradiated females (20 hairless and 19 haired) did not develop any tumors. The data on tumors found in untreated strain HR mice have been published previously (*1*) but are included in table 1 for the purpose of comparison. Both the hairless and haired males of the irradiated groups died earlier than the females probably because of damage to the kidneys following accidental exposure to chloroform (*2*). Sixty-six of 73 males in the present experiment have shown calcification of the kidney cortex in varying degrees. The fact that the males died at an earlier age than the females, however, did not affect the conclusions of this study since in the comparison of irradiated groups with control groups the data of the males and those of the females were kept separate. Two different types of tumors were found in 2 hairless males, in 4 hairless females, and in 10 haired females; however, there was no significant association between the types found. In the males the associated tumors were a hepatoma and a hemangioendothelioma in each case. In the females the associated tumors were one of the 3 types of ovarian tumors and any one of the 8 types of tumors listed. One haired female developed a lymphocytic neoplasm, a hemangioendothelioma, and an ovarian tumor.

In the present experiment the incidence of papillomas of the skin was not greater in the groups exposed to 400 r X radiation than that in the untreated groups. However, the papillomas which appeared in the irradiated group did so at an earlier average age than in the untreated group. (The average for the irradiated group was 10.8 $\pm$ 1.4 months and that for the untreated group was 19.0 $\pm$ 2.21 months. The difference between the two averages is statistically significant: $P<0.02$.) Squamous-cell carcinomas of the skin were observed in a small number of the irradiated hairless animals but were not observed in any of the irradiated haired animals. Following painting with 20-methylcholanthrene (*1*), all the animals of the HR strain developed papillomas, and transformation of the papillomas to carcinomas occurred in approximately 75 percent of the painted animals. A significantly higher incidence of carcinomas was found in the hairless than in the haired animals. The failure of X radiation to increase the incidence of papillomas differs from the effect of 20-methylcholanthrene.

The principal observed effect of the exposure of strain HR females to 400 r X radiation was damage to the ovaries. Twenty-two (15 haired

[5] The author is indebted to Dr. Thelma B. Dunn, Pathologist of the National Cancer Institute, who confirmed the diagnosis of many of the lesions observed in the animals of this experiment.

and 7 hairless) of 89 irradiated females developed ovarian tumors (granulosa-cell tumors, tubular adenomas, and a luteoma), whereas only one (haired) of 49 untreated females developed these tumors. One, or both, of the ovaries of 11 of the irradiated females were composed largely of luteal-like tissue; in some of them downgrowths of the germinal epithelium were observed. The ovaries of 3 of the animals were reduced in size and contained a few immature but no mature follicles. The ovaries of the remaining irradiated animals were cystic. The cysts contained hemorrhagic fluid in some cases but in the majority of cases the fluid was clear. In a few of the cystic ovaries a small amount of ovarian tissue with marked epithelial downgrowths persisted at the margin of the cyst and in others papillary projections into the cysts were noted.

An increase in the incidence of ovarian tumors fifteen times that of the controls was noted by Furth and Furth (*4*) in mice of stocks A, R, and S exposed to single or repeated doses of X rays ranging from 200 r to 400 r when 5 to 12 weeks old. Furth and Butterworth (*5*) have classified the ovarian tumors found following such exposures as granulosa-cell tumors, luteomata (regarded as a variety of granulosa-cell tumors), tubular adenomata, and such miscellaneous types as fibroma-like ovarian growth and papillary cystadenoma. These same types of ovarian tumors have been noted by various workers (6–9) in mice that had been exposed to radiation. Eschenbrenner (*10*) found the following types of tumors in LAF_1 hybrid mice following *gamma* irradiation: tubular adenomas, granulosa-cell tumors, luteomas, mixed tumors, and cystadenomas. The latter, though listed with the tumors, were not considered true neoplasms. All these types of ovarian tumors were seen in the ovaries of groups of LAF_1 hybrid females which had received total doses of 770 r and 880 r (*11*).

Different histologic stages in the vaginal epithelium indicating an estrous cycle were noted microscopically in some of the irradiated strain HR females. Parkes (*12*) stated that "all the normal cyclic phenomena can occur in animals in which the Graafian follicles of the ovary have been totally destroyed at an early age by exposure to x-rays, and can occur in the absence of organized corpora lutea and Graafian follicles." A similar persistence of estrous stages was noted in LAF_1 hybrid female mice receiving total doses of 770 r and 880 r (*11*).

Comparable with the effects on the ovaries of female mice of the HR strain, the principal effect of exposing males to 400 r was damage to the testes. In most of the animals, the testes were reduced in size. In almost all cases there was destruction of some of the seminiferous tubules and in some cases all of the tubules were damaged with only Sertoli cells remaining around the periphery of the tubules. In those tubules in which spermatogenesis persisted, the number of mature spermatozoa was usually reduced. These observations are consistent with those of Eschenbrenner, Miller, and Lorenz (*13*), who found a decrease in the spermatogenic elements in the testes of irradiated LAF_1 hybrid mice. Exposure to 8.8 r daily for 6 months or more resulted in failure to complete spermato-

TABLE 1.—*Tumors found in strain HR mice receiving 400 r X radiation at birth and in untreated strain HR mice*

Genotype and sex	Type of tumor	Irradiated mice				Untreated mice			
		Total number	Average age at death	Mice with tumor		Total number	Average age at death	Mice with tumor	
				Number	Average age at death			Number	Average age at death
		45	*Months* 13. 9		*Months*	25	*Months* 21. 6		*Months*
Hr/hr (haired) ♀ ♀	Papilloma of skin			1	8. 0				
	Pulmonary tumor			4	15. 5			4	24. 5
	Hemangioendothelioma			5	13. 4			3	21. 3
	Hepatoma			3	15. 3			3	23. 3
	Lymphocytic neoplasm*			6	13. 5			2	23. 0
	Reticulum-cell neoplasm—Type A*							1	19. 0
	Reticulum-cell neoplasm—Type B*			2	16. 5			3	17. 3
	Granulosa-cell tumor			10	12. 5			1	28. 0
	Tubular adenoma of ovary			4	15. 8				
	Luteoma			1	20. 0				
	Squamous-cell carcinoma of vagina			1	18. 0				
	Undiagnosed tumor (subcutaneous)			1	12. 0				
	Sarcoma of uterus							1	18. 0
	Harderian-gland tumor							1	28. 0
		44	15. 0			24	22. 3		
hr/hr (hairless) ♀ ♀	Papilloma of skin			2	13. 5			2	22. 5
	Squamous-cell carcinoma of skin			3	10. 7				
	Fibrosarcoma of skin			2	8. 5			1	23. 0
	Mammary-gland tumor			2	10. 0			1	18. 0
	Pulmonary tumor			2	16. 5				
	Hemangioendothelioma			5	17. 2			9	22. 1
	Hepatoma							3	26. 0
	Lymphocytic neoplasm			1	7. 0			1	21. 0
	Reticulum-cell neoplasm—Type A							1	15. 0
	Reticulum-cell neoplasm—Type B			2	17. 5			2	26. 5
	Granulosa-cell tumor			1	7. 0				
	Tubular adenoma of ovary			6	15. 5				

	Granulocytic leukemia			1	15.0			.....	.....
	Pituitary adenoma			1	14.0			.....	.....
Hr/hr (haired) ♂♂		41	9.5			28	19.4		
	Papilloma of skin			.....	.....			1	21.0
	Pulmonary tumor			.....	.....			1	23.0
	Hemangioendothelioma			.....	.....			7	20.0
	Hepatoma			4	13.0			6	21.6
	Lymphocytic neoplasm			2	6.0			3	19.3
	Reticulum-cell neoplasm—Type A			.....	.....			1	20.0
	Undiagnosed tumor of lymph node (resembles carcinoma).			1	13.0			.....	.....
hr/hr (hairless) ♂♂		32	11.5			21	19.9		
	Papilloma of skin			3	11.3			4	22.5
	Squamous-cell carcinoma			2	12.0			.....	.....
	Fibrosarcoma of skin			.....	.....			1	22.0
	Pulmonary tumor			.....	.....			1	26.0
	Hemangioendothelioma			3	12.3			3	23.0
	Hepatoma			5	14.0			3	24.3
	Lymphocytic neoplasm			1	8.0			2	14.5
	Reticulum-cell neoplasm—Type A			.....	.....			1	25.0

*This terminology follows the classification outlined by Thelma B. Dunn (3).

genesis, associated with a degeneration of Sertoli cells. The degenerating Sertoli cells were seen in masses in the lumen of the tubules. Such masses of cells were observed in some of the tubules in a few of the males in the present experiment. Eschenbrenner and Miller (*10*) found that the tubules were lined almost entirely by Sertoli cells in LAF_1 hybrid mice exposed to 8.8 r per 8-hour day and receiving total doses of 2,400 r.

Another observation in the irradiated strain HR mice was a lesion having some of the histologic features of periarteritis nodosa in the walls of the blood vessels in the testes. The endothelial lining cells were generally missing, and the vessels were lined and partly obstructed by an amorphous, eosinophilic, fibrinoid material. This appeared in 14 of the 70 males from which sections of the testis were available.

Lesions of the lens and retina, similar in many respects to those observed by Lorenz and Dunn (*14*) in the eyes of irradiated strain A mice, were observed in the eyes of the strain HR mice.

Summary

A total dose of 400 r X radiation failed to increase the incidence of papillomas of the skin in strain HR mice, but a decrease in the average age of appearance of the papillomas was noted. A few squamous-cell carcinomas were observed in the irradiated hairless (*hr*/*hr*) animals.

The number of ovarian tumors was increased in irradiated strain HR females as compared with that in control females.

Damage to the ovaries and testes of the strain HR animals was the principal result of the exposure to a total dose of 400 r X radiation within 12 hours following birth.

References

(*1*) Deringer, M. K.: Spontaneous and induced tumors in haired and hairless strain HR mice. J. Nat. Cancer Inst. 12: 437–445, 1951.

(*2*) Deringer, M. K., Dunn, T. B., and Heston, W. E.: Results of exposure of strain C3H mice to chloroform. Proc. Soc. Exper. Biol. & Med. 83: 474–479, 1953.

(*3*) Dunn, T. B.: Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J. Nat. Cancer Inst. 14: 1281–1433, 1954.

(*4*) Furth, J., and Furth, O. B.: Neoplastic diseases produced in mice by general irradiation with x-rays. I. Incidence and types of neoplasms. Am. J. Cancer 28: 54–65, 1936.

(*5*) Furth, J., and Butterworth, J. S.: Neoplastic diseases occurring among mice subjected to general irradiation with x-rays. II. Ovarian tumors and associated lesions. Am. J. Cancer 28: 66–95, 1936.

(*6*) Butterworth, J. S.: Observations on the histogenesis of ovarian tumors produced in mice by x-rays. Am. J. Cancer 31: 85–99, 1937.

(*7*) Geist, S. H., Gaines, J. A., and Pollack, A. D.: Experimental biologically active ovarian tumors in mice. Histogenesis and relationship to similar human ovarian tumors. Am. J. Obst. & Gynec. 38: 786–797, 1939.

(*8*) Lick, L., Kirschbaum, A., and Mixer, H.: Mechanism of induction of ovarian tumors by x-rays. Cancer Res. 9: 532–536, 1949.

(*9*) Lorenz, E.: Some biologic effects of long continued irradiation. Am. J. Roentgenol. 63: 176–185, 1950.

(*10*) ESCHENBRENNER, A., and MILLER, E.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. V. Pathological observations. *In* Biological Effects of External X and Gamma Radiation. Part I (Zirkle, R. E., ed.). New York, McGraw-Hill Book Co., 1954.

(*11*) DERINGER, M. K., HESTON, W. E., and LORENZ, E.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. IV. Actions on the breeding behavior of mice. *In* Biological Effects of External X and Gamma Radiation. Part I (Zirkle, R. E., ed.), NNES IV–22B, New York, McGraw-Hill Book Co., 1954, pp. 149–168.

(*12*) PARKES, A. S.: On the occurrence of the oestrous cycle after x-ray sterilisation. Part II. Irradiation at or before birth. Proc. Roy. Soc. s.B, 101: 71–94, 1927.

(*13*) ESCHENBRENNER, A. B., MILLER, E., and LORENZ, E.: Quantitative histologic analysis of the effect of chronic whole-body irradiation with gamma rays on the spermatogenic elements and the interstitial tissue of the testes of mice. J. Nat. Cancer Inst. 9: 133–147, 1948.

(*14*) LORENZ, E., and DUNN, T. B.: Ocular lesions induced by acute exposure of the whole body of newborn mice to roentgen radiation. Arch. Ophth. 43: 742–749, 1950.

Fertility and Tumor Development in (C57L × A)F_1 Hybrid Mice Receiving X Radiation to Ovaries Only, to Whole Body, and to Whole Body with Ovaries Shielded[1]

MARGARET K. DERINGER, EGON LORENZ,[2] *and* DELTA E. UPHOFF, *National Cancer Institute,*[3] *Bethesda, Md.*

Studies on the breeding behavior of LAF_1 hybrid mice exposed to long-continued total-body *gamma* irradiation have shown that female mice exposed to 770 and 880 r at rates of 8.8 r per 24-hour day, 4.4 r per 24-hour day and 8.8 r per 8-hour day exhibited sterility effects, with some of the animals completely sterile and with others producing litters of reduced size. Female mice exposed to 550 r at the rate of 4.4 r per 8-hour day bred but with reduced litter size (*1*). In order to extend these observations the present study on the breeding behavior of mice following X radiation to the ovaries only, to the whole body, to the whole body with ovaries shielded, and to the whole body with ovaries and spleen shielded has been made. The production of ovarian tumors following irradiation has been observed by numerous investigators (*1–6*). Whether such tumors are a result of a systemic effect of whole-body irradiation, or an effect on the ovaries only, or a combination of the two has been investigated (*7–9*) with various conclusions. An attempt has been made in the present investigation to add further information concerning the mechanism of the production of ovarian tumors following irradiation.

Materials and Methods

Genetically homogeneous (C57L × A)F_1 hybrid mice (formerly referred to as LAF_1 hybrid mice) were used as the experimental animals. These animals are first-generation hybrids between strain C57L females and strain A males. They are vigorous, long-lived animals and have a long breeding period producing as many as 10 to 14 litters in some cases.

Groups of female mice, consisting of 9 or 10 animals, were exposed at 2 months of age to total doses of 400, 300, 200, 100, 50, 25, and 12.5 r X radiation to the ovaries only, in one series, and to the whole body in another. An additional group was exposed to a total dose of 500 r to the ovaries only and two additional groups of 10 each were exposed to the 50 r

[1] Received for publication October 13, 1954.
[2] Deceased February 12, 1954.
[3] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

dose. The animals, receiving X radiation to the ovaries only, were protected during irradiation by a lead plate with a narrow slit that allowed exposure of the ovaries. The physical factors of irradiation were as follows: 186 KVP, 20 Ma., filtration with 0.25 mm. Cu and 0.55 mm. Al, distance from focus (single tube) to middle of mouse 50 cm., rate 60.4 r per minute. The exposed females were mated to untreated males immediately after irradiation. A group of 30 untreated females, which were mated to untreated males at 2 months of age, were maintained concurrently with the experimental animals.

In a group of 21 female (C57L $\times$ A)F_1 hybrid mice, the ovaries were surgically exteriorized and shielded by lead shields while the animals received a total dose of 400 r X radiation. A description of the ovary shield employed in these experiments and the technique for exteriorizing the ovary has been published by Wang (*10*). In a second group of 22 female mice, the ovaries and spleen were surgically exteriorized and put into lead shields while the animals received a total dose of 900 r X radiation. This technique is similar to that used by Jacobson and his associates (*11*), who were the first investigators to study recovery from irradiation injury utilizing the technique of spleen-shielding with lead following surgical exteriorization of the spleen. The physical factors of irradiation were as follows: 186 KVP, 20 Ma., filtration with 0.25 mm. Cu and 1.06 mm. Al, distance from focus (two tubes) to middle of mice 54 cm., rate 99 r per minute. The animals were allowed to recover from the effects of the operation before being mated. Consequently, the irradiated females were not mated to untreated males until 10 days following irradiation. In a control group of 22 mice the ovaries were surgically exteriorized and put into the lead shields for 5 minutes (a period of time comparable with that which elapsed during the exposure to X radiation) but without X radiation. The same procedure was followed for a control group of 21 mice in which the ovaries and spleen were put into the lead shields for 5 minutes without X radiation. Ten days later these two groups were mated to untreated males.

The animals were housed in wooden cages during the early months of the experiment but later were transferred to plastic cages. They were constantly supplied with Derwood food pellets and tap water. In all irradiated and control groups, the mice were subjected to a continuous breeding program wherein the females were isolated when pregnant and returned to the males at the weaning of each litter. When the animals became moribund or died, autopsies were performed, except for 24 animals in which extensive post-mortem autolysis had developed. The kidneys, testes, ovaries, uterus, and vagina were routinely taken for sectioning. Sections were also made of any tumor or any unusual lesion observed at autopsy. The tissues were fixed in Fekete's modification of Tellyesniczky's fixative (70 percent ethyl alcohol, 20 parts; formalin, 2 parts; glacial acetic acid, 1 part) and stained with hematoxylin and eosin.

Results and Discussion

The breeding results for the (C57L × A)F_1 hybrid mice receiving various total doses to the ovaries only and to the whole body are given in table 1, where it can be seen that at doses above 200 r, no female had more than one litter regardless of the site of irradiation. At the two lowest doses no abnormality in the breeding behavior was apparent. At intermediate doses the effect varied with the dosage and site of irradiation and was greatest in the case of whole-body irradiation. Females receiving total doses of 500, 400, and 300 r to the ovaries only produced one litter and were sterile thereafter. The average litter size in each group was reduced as compared with the average size of first litters of the untreated mice. Females receiving total doses of 200, 100 and 50 r to the ovaries only, produced multiple litters with the average size of the litters somewhat reduced as compared with that of the controls. The female mice which received total doses of 400, 300, 200, and 100 r to the whole body produced only one litter, the size of which was reduced in all groups, except in the 100 r group, as compared with the average size of first litters of the untreated animals. In the two groups receiving 50 r to the whole body, several litters were produced but all except the first litters were reduced in size. The groups receiving 25 and 12.5 r to the ovaries only and to the whole body bred comparably with the untreated group.

The breeding results obtained in the (C57L × A)F_1 hybrid mice receiving total doses of 400 r with the ovaries shielded and total doses of 900 r with the ovaries and spleen shielded are given in table 2. The animals receiving 400 r were not different from the control group in which the ovaries were put into the lead shields for 5 minutes without irradiation, whereas those receiving 900 r produced litters of slightly reduced size as compared with the control group in which the ovaries and spleen were put into the shields for 5 minutes without irradiation.

Graying of the hair, which has been described previously (*12–14*), was observed in the irradiated animals receiving total doses of 300 r and above in this experiment. There was a narrow band of gray hair on the back over the region of the ovaries in the females in which only the ovary was irradiated. Diffuse, irregularly-distributed graying of various degrees was observed in the animals which received X radiation to the whole body.

The principal effect of the X radiation was the damage to the exposed ovaries which resulted in tumors in the females receiving the higher doses. The incidence of ovarian tumors in the irradiated females is given in table 3. Other types of tumors were found to occur in irradiated and untreated female (C57L × A)F_1 hybrid mice. These tumors did not occur in numbers sufficient to establish an induction effect of the irradiation. The data on these lesions are presented in tables 4 and 5.[4] The terminology used for neoplasms of the reticular tissue follows the classification outlined by Dunn (*15*).

[4] The authors are grateful to Dr. Thelma B. Dunn, Pathologist of the National Cancer Institute, who confirmed the diagnosis of many of the lesions observed in the animals of this experiment.

TABLE 1.—*Average litter size born to (C57L × A)F_1 hybrid females*

Dose	Site of exposure	Total number irradiated	Average litter size for successive litters*				
500 r	Ovaries only	10	4. 4(9)				
400 r	Ovaries only	10	5. 4(10)				
	Whole body	10	4. 3(9)				
300 r	Ovaries only	9	5. 9(9)				
	Whole body	9	4. 8(9)				
200 r	Ovaries only	10	6. 5(10)	6. 6(5)	9. 0(4)	8. 2(4)	7. 2(4)
	Whole body	9	6. 6(8)				
100 r	Ovaries only	10	6. 0(10)	6. 4(8)	8. 5(8)	8. 0(8)	8. 0(8)
	Whole body	10	8. 0(8)				
50 r Group 1	Ovaries only	10	8. 2(10)	7. 8(10)	6. 1(10)	5. 9(9)	7. 0(6)
	Whole body	10	8. 0(10)	2. 7(10)	3. 0(3)	4. 0(2)	2. 0(1)
50 r Group 2	Ovaries only	10	8. 1(10)	7. 0(9)	8. 7(6)	7. 0(6)	5. 8(5)
	Whole body	10	8. 1(10)	1. 5(2)			
25 r	Ovaries only	10	9. 3(10)	9. 6(10)	10. 1(10)	9. 5(10)	9. 3(9)
	Whole body	10	8. 8(10)	10. 3(10)	10. 0(10)	8. 6(10)	9. 6(9)
12.5 r	Ovaries only	10	8. 5(10)	9. 1(10)	9. 7(10)	10. 3(10)	9. 6(10)
	Whole body	10	8. 4(10)	9. 3(10)	9. 8(10)	9. 3(10)	9. 1(8)
Untreated		30	7. 8(30)	9. 7(30)	10. 3(30)	9. 4(30)	9. 8(29)

*The figures in parentheses indicate the number of mothers contributing to the averages shown.

TABLE 2.—*Average litter size born to (C57L × A)F_1 hybrid females*

Dose	Organ shielded	Total number irradiated	Average litter size for successive litters *		
400 r	Ovaries	21	6. 2(21)	7. 8(21)	7. 0(20)
900 r	Ovaries and spleen.	22	4. 4(20)	4. 8(18)	4. 7(18)
Untreated	Ovaries	22	6. 4(22)	7. 5(21)	7. 7(21)
Untreated	Ovaries and spleen.	21	6. 0(20)	6. 3(20)	5. 8(20)

* The figures in parentheses indicate the number of mothers contributing to the averages shown.

receiving X radiation to the ovaries only and to the whole body

Average litter size for successive litters*—Continued								
4. 2(4)	5. 0(1)	6. 0(1)	5. 0(1)					
6. 4(7)	6. 1(7)	6. 8(5)	3. 3(3)	4. 0(2)				
7. 0(5)	5. 8(5)	6. 0(2)						
5. 5(2)	8. 0(2)	7. 0(1)	2. 0(1)					
10. 4(7)	8. 3(6)	5. 0(5)	6. 5(2)	8. 0(1)	3. 0(1)			
5. 6(8)	7. 4(5)	4. 5(4)	3. 0(1)	2. 0(1)				
10. 4(10)	8. 7(10)	6. 1(9)	5. 3(9)	4. 0(3)	2. 0(2)			
8. 1(8)	8. 0(8)	7. 1(6)	6. 7(3)					
9. 1(29)	8. 9(26)	7. 6(22)	6. 0(21)	4. 5(17)	2. 9(9)	2. 0(1)	5. 0(1)	2. 0(1)

receiving X radiati on with ovaries shielded and with ovaries and spleen shielded

Average litter size for successive litters *—Continued						
7. 3(19)	6. 8(15)	6. 5(12)	5. 1(7)	3. 2(4)	1. 0(1)	
4. 4(18)	3. 3(11)	2. 4(9)	3. 0(4)	4. 5(2)	3. 0(1)	
6. 7(20)	7. 1(18)	6. 9(16)	6. 8(11)	6. 4(8)	3. 8(4)	
5. 6(20)	5. 6(19)	5. 2(19)	5. 1(16)	4. 0(11)	5. 0(5)	3. 0(1)

TABLE 3.—*Incidence of ovarian tumors in irradiated and control (C57L × A)F_1 hybrid mice*

Treatment	Total number of mice	Average age at death	Mice with ovarian tumors	
			Number	Average age at death
		Months		*Months*
500 r ovaries only	9	16. 8	9	16. 8
400 r ovaries only	8	17. 8	8	17. 8
400 r whole body	8	21. 4	8	21. 4
300 r ovaries only	9	17. 4	9	17. 4
300 r whole body	8	19. 3	8	19. 3
200 r ovaries only	8	19. 9	4	22. 5
200 r whole body	9	19. 0	9	19. 0
100 r ovaries only	10	21. 2	3	21. 6
100 r whole body	10	23. 3	9	24. 9
50 r ovaries only	18	19. 2	5	24. 6
50 r whole body	17	18. 9	12	21. 3
25 r ovaries only	10	17. 0	0	——
25 r whole body	10	19. 4	0	——
12.5 r ovaries only	10	21. 5	0	——
12.5 r whole body	10	16. 4	0	——
Untreated	24	21. 6	0	——
Ovaries shielded + 400 r	19	17. 5	0	——
Ovaries and spleen shielded + 900 r	21	14. 5	0	——
Ovaries shielded	21	19. 3	0	——
Ovaries and spleen shielded	20	21. 9	0	——

TABLE 4.—*Types of tumors found in irradiated and control female (C57L × A)F_1 hybrid mice*

Dose	Total number of mice	Average age at death	Type of tumor	Mice with tumor	
				Number	Average age at death
		Months			*Months*
500 r ovaries only.	9	16. 8	Hepatoma	1	22. 0
			Polyp of uterus	1	17. 0
400 r ovaries only.	8	17. 8	Pulmonary tumor	2	17. 5
400 r whole body.	8	21. 4	Hepatoma	1	22. 0
			Tumor of adrenal medulla	1	22. 0
			Lymphocytic neoplasm	1	20. 0
			Unusual uterine tumor	1	22. 0
			Pulmonary tumor	4	21. 5
300 r ovaries only.	9	17. 4	Tumor of adrenal cortex	1	18. 0
300 r whole body.	8	19. 3	Reticulum-cell neoplasm—Type A.	1	16. 0
			Reticulum-cell neoplasm—Type B.	1	20. 0
			Polyp of uterus	1	16. 0
			Adenocarcinoma of uterus	1	22. 0
			Pulmonary tumor	2	20. 5

TABLE 4.—*Types of tumors found in irradiated and control female (C57L × A)F_1 hybrid mice*—Continued

Dose	Total number of mice	Average age at death	Type of tumor	Mice with tumor	
				Number	Average age at death
		Months			*Months*
200 r ovaries only.	8	19. 9	Hepatoma	1	27. 0
			Lymphocytic neoplasm	1	26. 0
			Fibrosarcoma (in region of mesenteric node).	1	11. 0
			Pulmonary tumor	3	24. 0
200 r whole body.	9	19. 0	Reticulum-cell neoplasm—Type A.	2	16. 0
			Pulmonary tumor	3	20. 6
			Mammary gland tumor (Type C)	1	18. 0
100 r ovaries only.	10	21. 2	Hepatoma	1	19. 0
			Reticulum-cell neoplasm—Type B.	2	21. 5
			Fibromyoma of uterus	2	23. 0
			Pulmonary tumor	2	18. 0
			Subcutaneous tumor (fusiform cell)	1	23. 0
100 r whole body.	10	23. 3	Hepatoma	2	33. 5
			Tumor of adrenal medulla	1	23. 0
			Reticulum-cell neoplasm—Type B.	7	23. 7
			Polyp of uterus	1	21. 0
			Adenocarcinoma of uterus	1	24. 0
			Pulmonary tumor	2	28. 0
			Mast-cell tumor (subcutaneous)	1	23. 0
50 r ovaries only.	18	19. 2	Hepatoma	2	24. 0
			Reticulum-cell neoplasm—Type B.	5	23. 4
			Fibrosarcoma (subcutaneous)	1	23. 0
			Pulmonary tumor	4	23. 0
50 r whole body.	17	18. 9	Hepatoma	2	20. 0
			Reticulum-cell neoplasm—Type B.	4	21. 8
			Fibrosarcoma (subcutaneous)	1	19. 0
			Pulmonary tumor	8	19. 8
			Squamous-cell carcinoma of skin	1	8. 0
			Undiagnosed tumor of lymph node	1	20. 0
25 r ovaries only.	10	17. 0	Reticulum-cell neoplasm—Type B.	1	21. 0
			Fibromyoma of uterus	2	23. 5
			Pulmonary tumor	2	19. 0
25 r whole body.	10	19. 4	Reticulum-cell neoplasm—Type A.	1	20. 0
			Reticulum-cell neoplasm—Type B.	2	22. 0
			Pulmonary tumor	3	23. 3
12.5 r ovaries only.	10	21. 5	Anaplastic carcinoma of liver	1	24. 0
			Lymphocytic neoplasm	2	21. 0
			Reticulum-cell neoplasm—Type B.	1	22. 0
			Pulmonary tumor	6	21. 8
12.5 r whole body.	10	16. 4	Hepatoma	1	24. 0
			Fibromyoma of uterus	1	19. 0
			Fibrosarcoma of uterus	1	15. 0
			Pulmonary tumor	5	15. 4
Untreated.	24	21. 6	Hepatoma	3	22. 3
			Reticulum-cell neoplasm—Type B.	6	23. 3
			Pulmonary tumor	12	21. 8

TABLE 5.—*Types of tumors found in (C57L × A)F_1 hybrid females receiving 400 r total-body X radiation with ovaries shielded, 900 r total-body X radiation with ovaries and spleen shielded, and in controls*

Treatment	Total number of mice	Average age at death	Type of tumor	Mice with tumors	
				Number	Average age at death
		Months			*Months*
Ovaries shielded + 400 r.	19	17. 5	Hepatoma	3	20. 0
			Lymphocytic neoplasm	1	8. 0
			Reticulum-cell neoplasm—Type A	2	21. 0
			Reticulum-cell neoplasm—Type B	5	23. 6
			Polyp of uterus	1	25. 0
			Fibrosarcoma of uterus	1	24. 0
			Pulmonary tumor	8	23. 0
			Mammary tumor (1 miscellaneous, 1 molluscoidal).	2	9. 5
			Squamous-cell carcinoma of skin	1	13. 0
			Malignant lymphoma (cell type undetermined).	1	22. 0
Ovaries and spleen shielded + 900 r.	21	14. 5	Reticulum-cell neoplasm—Type B	1	18. 0
			Pulmonary tumor	1	10. 0
			Mammary tumor (Type C)	1	17. 0
Ovaries shielded	21	19. 3	Hepatoma	3	23. 0
			Reticulum-cell neoplasm—Type A	1	28. 0
			Reticulum-cell neoplasm—Type B	2	17. 5
			Fibromyoma of uterus	1	28. 0
			Pulmonary tumor	4	20. 5
Ovaries and spleen shielded.	20	21. 9	Hepatoma	1	31. 0
			Reticulum-cell neoplasm—Type A	2	19. 5
			Reticulum-cell neoplasm—Type B	4	22. 2
			Pulmonary tumor	6	25. 2
			Squamous-cell carcinoma of skin	1	16. 0

Ovarian tumors occurred bilaterally in all of the animals (for which histologic sections were available) receiving total doses of 500, 400, and 300 r to the ovaries only and total doses of 400, 300, 200, and 100 r (except for one animal) to the whole body including the ovaries. In most cases the ovarian tumors were mixed tumors. Granulosa-cell tumors, luteomas, and tubular adenomas have been observed singly and in combinations of all of the three types. Often one or two of these types were seen in combination with cysts filled with clear fluid, with cysts filled with bloody fluid, with cysts containing papillary projections, or with papillary cystadenomas. Ovarian tumors were observed in some of the females receiving 200, 100, and 50 r to the ovaries only and in females receiving 50 r to the whole body. The ovaries in the remaining animals of these groups were reduced in size, composed largely of luteal-like tissue, with occasional growing follicles, and with marked downgrowths of the germinal epithelium. Occasionally the ovary had been replaced by a cyst. No ovarian tumors were observed in any of the females receiving 25 and 12.5 r

to the ovaries only and to the whole body. The ovaries of these animals were normal in appearance in some cases, in others they were generally reduced in size but with growing follicles present. In some cases mature follicles were observed. Downgrowths of the germinal epithelium were sometimes observed and in some cases the ovaries were cystic. This was similar to control mice where these same observations have been made in the untreated females and in all the animals (for which sections were available) in the shielding experiment.

Infiltration of mast cells into the ovarian tumors has been observed in some of the animals receiving total doses of 500, 400, 300, 200, 100, and 50 r to the ovaries only and in some of the animals receiving total doses of 400, 300, 200, 100, and 50 r to the whole body. It was also observed in the ovaries of two of the animals receiving a total dose of 25 r to the whole body. Bali and Furth (*16*) found mast cells in a number of neoplasms, among which were both spontaneous and transplanted luteomas; however, they failed to find mast cells in granulosa-cell tumors.

A lesion, similar to periarteritis nodosa, was observed in the blood vessels of the ovary in a few females receiving total doses of 25 r to the ovaries only, 25 and 12.5 r to the whole body and in females receiving total doses of 900 r with ovaries and spleen shielded. This lesion has recently been observed in the blood vessels of the testes of strain HR mice which received a total dose of 400 r X radiation following birth (*17*).

Adenomyosis, a condition observed by Christy, Dickie, Atkinson, and Woolley (*18*) in the uteri of virgin and castrate reciprocal F_1 hybrid females between strains DBA and CE, was observed in the uteri of some of the animals receiving total doses of 500, 300, and 50 r to the ovaries only, total doses of 400, 200, 100, and 50 r to the whole body, and in one animal receiving a total dose of 900 r with ovaries and spleen shielded.

In some of the females of all of the irradiated groups the existence of an estrous cycle was indicated by various histologic stages noted microscopically in the vaginal epithelium. A similar observation was made in strain HR females receiving 400 r immediately following birth (*17*) and in LAF_1 hybrid mice receiving total doses of 770 and 880 r (*1*). Parkes (*19*) has observed that all normal cyclic manifestations of estrus except ovulation can occur in animals in which the follicles have been destroyed by X rays and in which follicles and corpora lutea are absent. In animals in which the existence of an estrous cycle was not observed, the vaginal epithelium was atrophied in some cases and in others mucinous cells were present.

Summary

Female (C57L × A)F_1 hybrid mice in which the ovaries only were exposed to total doses of 300 to 500 r produced one litter and were sterile thereafter. Litter size was reduced as compared with that of untreated mice.

Females in which the ovaries only were exposed to total doses of 50 to 200 r produced multiple litters of litter size somewhat less as compared with untreated mice.

Females receiving total doses of 100 to 400 r to the whole body produced only one litter with reduced litter size, except in the 100 r group, as compared with untreated mice.

Females receiving total doses of 12.5 to 50 r to either site produced multiple litters of comparable size with the untreated animals in the 12.5 and 25 r groups but with reduced litter size, as compared with the untreated animals in the 50 r groups.

The breeding behavior of animals receiving 400 r with the ovaries shielded was similar to that of the control group. In animals receiving 900 r with the ovaries and spleen shielded the average litter size was slightly reduced as compared with that of the controls.

Ovarian tumors occurred in all the animals receiving total doses of 300 to 500 r to the ovaries only and total doses of 100 to 400 r to the whole body, and these tumors also occurred in some of the females receiving total doses of 50 to 200 r to the ovaries only and in some of those receiving a total dose of 50 r to the whole body.

The greatest effect of X radiation was noted when animals received irradiation to the whole body, a lesser effect when ovaries only were irradiated, and the least effect when the whole body was irradiated with the ovaries shielded. Although the greatest effect was obtained when the whole body was irradiated, it was necessary that the irradiation include the ovaries. No effect was apparent when the whole body excluding the ovaries was irradiated other than that which could be ascribed to the operation itself.

References

(*1*) Deringer, M. K., Heston, W. E., and Lorenz, E.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. IV. Actions on the breeding behavior of mice. *In* Biological Effects of External X and Gamma Radiation. Part I (Zirkle, R. E., ed.) NNES IV–22B, New York, McGraw-Hill Book Co., 1954, pp. 149–168.

(*2*) Eschenbrenner, A. B., and Miller, E.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. V. Pathological observations. *In* Biological Effects of External X and Gamma Radiation. Part I (Zirkle, R. E., ed.). New York, McGraw-Hill Book Co., 1954.

(*3*) Furth, J., and Furth, O. B.: Neoplastic diseases produced in mice by general irradiation with x-rays. I. Incidence and types of neoplasms. Am. J. Cancer 28: 54–65, 1936.

(*4*) Furth, J., and Butterworth, J. S.: Neoplastic diseases occurring among mice subjected to general irradiation with x-rays. II. Ovarian tumors and associated lesions. Am. J. Cancer 28: 66–95, 1936.

(*5*) Butterworth, J. S.: Observations on the histogenesis of ovarian tumors produced in mice by x-rays. Am. J. Cancer 31: 85–99, 1937.

(*6*) Geist, S. H., Gaines, J. A., and Pollack, A. D.: Experimental biologically active ovarian tumors in mice. Histogenesis and relationship to similar human ovarian tumors. Am. J. Obst. & Gynec. 38: 786–797, 1939.

(*7*) Furth, J., and Boon, M. C.: Induction of ovarian tumors in mice by x-rays. Cancer Res. 7: 241–245, 1947.

(*8*) Lick, L., Kirschbaum, A., and Mixer, H.: Mechanism of induction of ovarian tumors by x-rays. Cancer Res. 9: 532–536, 1949.

(*9*) Kaplan, H. S.: Influence of ovarian function on incidence of radiation induced ovarian tumors in mice. J. Nat. Cancer Inst. 11: 125–132, 1950.

(*10*) WANG, T. J.: Selective shielding against roentgen radiation. Radiology 61: 401–404, 1953.

(*11*) JACOBSON, L. O., MARKS, E. K., GASTON, E. O., ROBSON, M. J., and ZIRKLE, R. E.: The role of the spleen in radiation injury. Proc. Soc. Exper. Biol. & Med. 70: 740–742, 1949.

(*12*) HANCE, R. T., and MURPHY, J. B.: Studies on x-ray effects. XV. The prevention of pigment formation in the hair follicles of colored mice with high voltage x-ray. J. Exper. Med. 44: 339–342, 1926.

(*13*) CHASE, H. B.: Greying of hair. I. Effects produced by single doses of x-rays on mice. J. Morphol. 84: 57–79, 1949.

(*14*) BOYLAND, E., and SARGENT, S.: The local greying of hair in mice treated with x rays and radiomimetic drugs. Brit. J. Cancer 5: 433–440, 1951.

(*15*) DUNN, T. B.: Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J. Nat. Cancer Inst. 14: 1281–1433, 1954.

(*16*) BALI, T. and FURTH, J.: A transplantable splenic tumor rich in mast cells. Observations on mast cells in varied neoplasms. Am. J. Path. 25: 605–625, 1949.

(*17*) DERINGER, M. K., and LORENZ, E.: Results of exposure of newborn strain HR mice to X radiation. J. Nat. Cancer Inst. 15: 923–929, 1955.

(*18*) CHRISTY, N. P., DICKIE, M. M., ATKINSON, W. B., and WOOLLEY, G. W.: The pathogenesis of uterine lesions in virgin mice and in gonadectomized mice bearing adrenal cortical and pituitary tumors. Cancer Res. 11: 413–422, 1951.

(*19*) PARKES, A. S.: On the occurrence of the oestrous cycle after x-ray sterilisation. II. Irradiation at or before birth. Proc. Roy. Soc., London, s.B, 101: 71–95, 1927.

Comparison of Thyroid Lobes, Autotransplanted and *In Situ*, in the Rat: Growth and I^{131} Uptake[1]

SEYMOUR H. WOLLMAN *and* ROBERT O. SCOW,[2]
National Cancer Institute and National Institute of Arthritis and Metabolic Diseases,[3] Bethesda, Md.

Thyroid tissue can be successfully transplanted to a variety of sites in many species. There is much evidence that the transplanted tissue retains many of the physiological properties of normal thyroid tissue. The transplanted tissue responds to stimulation by thyroid stimulating hormone (*1*, *2*) or by goitrogen feeding (*3*, *4*). It can also accumulate injected radioiodide (*2*, *5–11*).

However, there have been few reports of quantitative comparisons of transplanted normal thyroid tissue and thyroid tissue *in situ* with respect to functional activity (*2*) and growth in response to stimuli. The results of a limited investigation of these questions will be reported in this paper.

Materials and Methods

Autotransplantation.—Transplantation of thyroid tissue was performed on 1- or 2-day-old male Sprague-Dawley rats while under refrigeration anesthesia (*12*). When thyroid tissue was removed from the trachea, bleeding was controlled by use of a small electric cautery. When a single lobe was removed it was separated from the other lobe by dividing the isthmus in the midline. The tissue to be transplanted was inserted subcutaneously along the left lateral thoracic artery near the subcostal margin in the animal of origin. The left lobe alone was usually transplanted, although in a small number of animals the right lobe or both lobes were transplanted. The rats in each litter were randomized among the above three groups and an additional group in which both thyroid lobes were left *in situ*. After being warmed to room temperature, the rats were returned to their mothers. During the second week the number of rats in each litter was reduced to six. Rats were weaned at 30 days of age.

Diet.—All rats, including the mothers, were fed a lactating diet *ad libitum* (*13*). Propylthiouracil (2 gm./kg.) was added to the diet fed to certain

[1] Received for publication October 12, 1954.
[2] We are grateful to Franklin E. Reed for technical assistance.
[3] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

groups starting at 30 days of age. Three days before the radioiodine uptake studies, the propylthiouracil was removed from the diet.

Hypophysectomy.—Hypophysectomy was performed on some groups of animals when they were 30 days old. Completeness of extirpation was ascertained by visual inspection of the region of the sella turcica for pituitary fragments using a binocular microscope at 9× magnification.

At 30 or 47 days of age, rats were given intraperitoneal injections of 10 μc. of carrier-free radioiodide[4] in one-half ml. of distilled water. Two hours later, each rat was anesthetized with ether and exsanguinated. The thyroid lobes were excised separately, weighed and put into sample tubes containing formalin-Zenker. The I^{131} content of each lobe was measured using a well-type Geiger tube. Histologic sections were obtained at five representative levels from both thyroid lobes in half the rats in each group.

Results

Body weights.—In rats in which one or both thyroid lobes were autotransplanted to a subcutaneous site body weights were not different from those of rats in which both lobes were left *in situ* (table 1).

Thyroid weights.—When both thyroid lobes were *in situ* the left lobes weighed the same or only slightly less than the right lobes. When one lobe was autotransplanted, it weighed only 0.3 to 0.5 as much as the lobe *in situ*. If both lobes were transplanted the total weight of the lobes was greater than twice the weight of the transplanted lobe in the group in which only one lobe was transplanted. In hypophysectomized rats both lobes *in situ* and transplanted lobes atrophied slightly. After 2 weeks of propylthiouracil feeding both autotransplants and lobes *in situ* were enlarged to 3.5 to 4 times those of the corresponding groups fed the stock diet (table 1).

Thyroid histology.—In all cases autotransplants were histologically indistinguishable from thyroid lobes *in situ* in the same host. They exhibited flattened epithelial cells after hypophysectomy, and hyperplasia and hypertrophy with resorption of colloid after propylthiouracil feeding. No histologic evidence of necrosis was found in any transplant. Parathyroids persisted in the transplants as previously described by Ingle and Cragg (*14*).

Radioiodine uptake.—The uptake of radioiodine per mg. of thyroid tissue was the same for both lobes *in situ* in normal and propylthiouracil-fed rats, and slightly less for the left lobe than the right in the hypophysectomized rats. An autotransplanted lobe differed from a lobe *in situ* generally by less than 10 percent, except for the three hypophysectomized rats in which the autotransplanted lobe averaged 25 percent higher than the lobe *in situ* (table 1).

Discussion

The present results indicate no significant difference between thyroid lobes *in situ* and lobes which were autotransplanted to a subcutaneous site.

[4] The I^{131} was obtained from the Oak Ridge National Laboratory, Oak Ridge, Tenn.

TABLE 1.—*Comparison of autotransplanted lobes of thyroid gland with lobes* in situ: *weight and* I^{131} *uptake*

Group	Lobe transplanted	Number of rats	Rat weight (*gm.*)		Thyroid weight (*mg.*)			Weight transplant	Thyroid I^{131} (*percent dose*)*	I^{131}/mg. transplant
			30 days	47 days	Right	Left	Total	Weight lobe *in situ*		I^{131}/mg. lobe *in situ*
30 days old Normal...	None.	7	70± 7†	.....	3.3±0.4†	3.1±0.4†	6.4±0.5†	0.94±0.05‡§	.....	0.94±0.04‡§
	Left..	6	73±10	.....	4.2±0.8	1.8±0.4	6.0±1.2	0.39±0.02	.....	0.86±0.03
	Both..	4	66± 6	.....	.....	.....	4.3±0.8	.....	.....	.....
47 days old Normal...	None.	5	70±18	151±28†	5.9±1.2	5.0±0.8	10.8±2.0	0.85±0.03§	4.9 ±0.5‡	0.98±0.04§
	Left..	7	62±14	148±30	6.6±1.0	2.3±0.9	8.9±1.5	0.35±0.05	4.4 ±0.2	0.99±0.06
	Right.	4	60± 5	141±12	2.9±0.9	6.6±0.9	9.5±1.6	0.44±0.06	4.4 ±0.7	1.06±0.07
	Both..	9	62±12	148±26	.....	.....	7.8±2.3	.....	3.6 ±0.4	.....
Hypophysectomized	None.	4	79±11	85±10	3.0±0.3	2.6±0.5	5.6±0.5	0.87±0.07§	0.27±0.12	0.83±0.06§
	Left..	3	88±13	94± 7	4.2±0.7	1.3±0.3	5.5±0.8	0.31±0.03	0.18±0.07	1.25±0.14
Propylthiouracil‖	None.	8	72± 9	145±23	22.5±5.9	18.3±2.8	40.8±7.1	0.84±0.06§	18.1 ±2.3	0.98±0.02§
	Left..	7	58± 9	117±22	22.7±2.7	11.0±3.3	33.7±4.2	0.49±0.06	16.4 ±3.1	0.94±0.06

*Two hours after radioiodide injection. Sum of uptakes by both lobes.

†Standard deviation.

‡Standard error.

§If both lobes *in situ*, this ratio contains data for left lobe in numerator and for right lobe in denominator.

‖Fed propylthiouracil from 30th to 44th day of age; no goitrogen for 3 days before I^{131} uptake studies.

Thyroid lobes at both sites in the same rat grew to the same extent in response to propylthiouracil feeding; the uptake of radioiodine per mg. of tissue or the serum radioiodide clearance per mg. of tissue (*15*) was almost the same for the autotransplants and the lobes *in situ* (table 1). The present results appear to differ slightly from some preliminary observations (*16*). The difference may have occurred because too few rats were used in the preliminary experiments or, perhaps, because the I^{131} uptake was measured at 24 and 48 hours after radioiodide injection in the preliminary experiments instead of at 2 hours. In agreement with other investigations (*17, 18*), when both lobes were autotransplanted the rats grew at the same rate as intact rats, indicating an approximately normal rate of thyroxine production in the autotransplants.

It has been found that transplantable thyroid tumors differ from thyroid gland in the same mouse by growing faster than thyroid gland in response to thiouracil feeding and by clearing less radioiodide from the blood per unit weight of tissue (*16*). The present results in the rat suggest that the above changes are inherent in the tissue and are probably not due to the location of the tumor transplants in an ectopic site.

The transplanted lobe was always smaller than the lobe *in situ*. This size difference may be a consequence of some necrosis in the transplant shortly after transplantation. Such partial necrosis followed by early regeneration and recovery has been observed repeatedly by others (*5, 6, 14, 19, 20*).

Summary

A comparison was made of growth characteristics and radioiodide uptake of autotransplanted thyroid lobes and lobes *in situ* in the same rats. Although autotransplanted lobes were always smaller than lobes *in situ* their clearance of radioiodide from the blood was not significantly different per unit weight of tissue. No significant difference was found in their response to hypophysectomy or propylthiouracil feeding.

References

(*1*) Marine, D., and Rosen, S. H.: The effect of thyrotropic hormone on auto- and homeotransplants of the thyroid and its bearing on the question of secretory nerves. Am. J. Physiol. 107: 677–680, 1934.

(*2*) Dameron, J. T.: The effect of thyrotrophic hormone and propylthiouracil on homologous thyroid transplants. Surgical Forum 3: 681–685, 1953.

(*3*) Bielschowsky, F., Griesbach, W. E., Hall, W. H., Kennedy, T. H., and Purves, H. D.: Studies on experimental goitre: The transplantability of experimental thyroid tumours of the rat. Brit. J. Cancer 3: 541–546, 1949.

(*4*) Morris, H. P., and Green, C. D.: The role of thiouracil in the induction, growth, and transplantability of mouse thyroid tumors. Science 114: 44–46, 1951.

(*5*) Bennett, D., and Gorbman, A.: Reestablishment of function in transplanted thyroid glands of mice. Endocrinology 49: 310–317, 1951.

(*6*) Desaive, P., Mewissen, D., and Closon, J.: Etude des greffes thyroïdiennes intrasplèniques principalement au point de vue de leur réponse à l'iode radioactif I^{131}. J. Physiol., Paris 43: 703–712, 1951.

(7) Wollman, S. H., Morris, H. P., and Green, C. D.: Function of transplantable tumors of the thyroid gland in C3H mice. J. Nat. Cancer Inst. 12: 27–35, 1951.

(8) Purves, H. D., Griesbach, W. E., and Kennedy, T. H.: Studies in experimental goitre: Malignant change in a transplantable rat thyroid tumour. Brit. J. Cancer 5: 301–310, 1951.

(9) Bariatti, R.: Innesti sperimentale di tiroide controllati con isotopi radioattivi (I. 131). Sperimentale 103: 43–53, 1953.

(10) Marinoni, U., and Bonatelli, A.: L'innesto tiroides auto-omo-etero-plastico controllato con il radioiodio. Tumori 39: 189–198, 1953.

(11) Szilagyi, D. E., Barrett, J. L., Longabaugh, E. R., and Preuss, L. E.: A radioiodine tracer study of the fate of human thyroid autotransplants. J. Clin. Endocrinol. 13: 1347–1357, 1953.

(12) Scow, R. O., Simpson, M. E., Asling, C. W., Li, C. H., and Evans, H. M.: Response by the rat thyro-parathyroidectomized at birth to growth hormone and to thyroxin given separately or in combination. I. General growth and organ changes. Anat. Rec. 104: 455–463, 1949.

(13) Scow, R. O.: Effect of growth hormone on various protein fractions in striated muscle of thyroidectomized and hypophysectomized rats. Am. J. Physiol. 173: 199–206, 1953.

(14) Ingle, D. J., and Cragg, R. W.: The regeneration of autografts of thyroid tissue in partially and completely thyroidectomized rats. Endocrinology 24: 550–552, 1939.

(15) Wollman, S. H.: Radioiodine uptake by transplantable tumors of the thyroid gland in C3H mice. II. Theory. J. Nat. Cancer Inst. 13: 807–814, 1953.

(16) Wollman, S. H., Scow, R. O., Wagner, B., and Morris, H. P.: Radioiodine uptake by transplantable tumors of the thyroid gland in C3H mice. I. Experimental results. J. Nat. Cancer Inst. 13: 785–805, 1953.

(17) Bondy, P. K.: Maintenance of normal thyroid activity after transplantation of thyroid gland into spleen or kidney. Proc. Soc. Exper. Biol. & Med. 77: 638–640, 1951.

(18) Rupp, J. J.: Action of liver on thyroid hormone following intrasplenic implantation of the thyroid. Endocrinology 51: 306–310, 1952.

(19) de Pasqualini, C. D., and Mancini, R. E.: Greffe de thyroïde dans la rate. Compt. rend. Soc. biol. 145: 1719–1722, 1951.

(20) Brachetto-Brian, D., and Grinberg, R.: Processus histologique des autogreffes intraspléniques des thyroïdes chez les rats thyroïdectomisés. Compt. rend. Soc. biol. 146: 136–137, 1952.

Some Biological Aspects of the Factor in Bone Marrow Responsible for Hematopoietic Recovery Following Systemic Irradiation[1, 2, 3]

MARY B. BROWN, BARBARA B. HIRSCH, C. SUSAN NAGAREDA, SARAH K. HOCHSTETLER, WILLIAM G. FARAGHAN, PAUL TOCH, *and* HENRY S. KAPLAN, *Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.*

The work of Jacobson and his group (*1–3*), Lorenz and his colleagues (*4–7*), Cole *et al.* (*8*), the authors (*9–11*), and others (*12, 13*) has clearly established the existence of an unidentified material in bone marrow and spleen which prevents postirradiation death in mice and rabbits and inhibits the later development of radiation-induced lymphoid tumors in mice. Its action is not inherently prophylactic or protective, however, since marrow-injected animals suffer the same severe initial damage as control animals receiving the same dose of X rays. Instead, it appears to promote early regeneration of the hematopoietic tissues and thus to bring about the other effects mentioned.

Several attempts to extract this material in biologically active form, using a variety of media and extraction procedures, have failed (*3, 11*). In mouse marrow centrifugates, the activity was associated with the washed cells, and not with the supernate (*11*). However, Hilfinger and Ferguson (*14*) have recently reported that a cell-free extract of rabbit bone marrow exhibited significant activity against radiation mortality in rabbits. Using Schneider's method of differential centrifugation (*15*) and a modified sucrose-salt medium fortified with adenosine triphosphate, Cole, Fishler, and Bond (*8*) prepared and assayed subcellular fractions of mouse spleen cells. The cytoplasmic and supernatant fractions were inactive but the nuclei fraction, which was slightly contaminated with nondisrupted cells, was effective in reducing mortality when given intraperitoneally to mice exposed to total-body X ray doses in the LD_{50-100} range. The nuclei lost their activity when treated prior to injection with either desoxyribonuclease or trypsin, but were unaffected by incubation with ribonuclease. They concluded that the active material resides in the nucleus and is probably a desoxyribonucleoprotein, and they drew an analogy between its action on radiation-injured hematopoietic tissues and the mode of action of the bacterial transforming principle, which is known to be a desoxyribonucleic acid (*16, 17*).

[1] Received for publication October 27, 1954.

[2] Presented in part at the annual meeting of the Radiation Research Society, Cleveland, May 17 to 19, 1954.

[3] These studies were supported by grants-in-aid from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and the Jane Coffin Childs Memorial Fund for Medical Research.

It seemed that the difficult task of extracting and isolating the marrow principle in reasonably pure, cell-free form might be facilitated if the biological factors which modify its activity were better defined. Some of the biological characteristics of the material have been previously described. This paper presents the results of an extensive series of experiments intended to provide additional information along several lines.

Experimental Procedures and Results

The technical methods for the preparation of marrow cell suspensions, dose and periodicity of irradiation, and other procedures were identical with those previously described (*11*), except where otherwise indicated. As before, strain C57BL mice of both sexes, usually about 33 days old at the start of treatment, were used, and their thymic weight at experimental day 50 (25 days after the last of 4 fractional irradiations at 8-day intervals) served as the assay criterion. Other lymphoid-tissue weights were also determined, as in earlier experiments. Data are tabulated as group means ± standard errors [estimated as range estimate of the standard deviation divided by $\sqrt{n}$. (*18*)].

I. In vivo *"dialysis" of Marrow*

In two experiments, homologous bone-marrow cells suspended in sterile Locke's solution were sealed aseptically in special Teflon capsules[4] and inserted into the peritoneal cavities of groups of irradiated mice. The capsules each contained about 0.7 ml. of suspension. They were sealed at the loading end by a stainless-steel screw, and at the other end by a window of such porosity that bacteria and cells could not escape from the chamber but proteins, body fluids, and various solutes could freely move in and out. It was hoped that the marrow cells could remain viable for hours or perhaps days in this environment and continue to produce the active factor under circumstances where its ability to dissociate from its cell of origin might be demonstrable under critical conditions. Control groups received irradiation alone or capsules containing the suspending medium. As table 1 reveals, there was no evidence that a cell-free material having this activity escaped from the capsule chamber into the host in either experiment. Marrow suspensions injected directly into the peritoneal cavity were active, however, as had previously been demonstrated (*4*). The introduction of the capsule into the peritoneal cavity appeared to be quite traumatic for the irradiated mice. Thymic weights in the capsule-implanted animals, whether receiving marrow or Locke's solution, were even lower than those of the irradiated controls, and the general appearance of these animals, a few of which died during the two experiments, was poor. Cells were present in the capsules when they were removed after 3 weeks but were of dubious viability as judged by staining reactions. The omentum was found to be intimately applied to the window of the capsule. No bacterial growth from the intracapsular fluid was obtained in 48-hour cultures on agar slants and in beef broth at 37° C.

[4] These capsules were very kindly loaned to us by Dr. Sidney Raffel, Professor of Bacteriology, Stanford University.

TABLE 1.—*Effect of* in vivo *"dialysis" of bone marrow on lymphoid-tissue recovery in irradiated strain C57BL female mice*

Expt. No.	Treatment	No. of mice	Thymus (*mg.*)	Nodes (*mg.*)	Spleen (*mg.*)	Body weight (*mg.*)
1	X ray only	6	29.0 ± 6.3	5.7 ± 0.8	75.4 ± 3.0	18.8 ± 0.4
	X ray + capsule + marrow	6	21.6 ± 4.8	8.3 ± 2.0	91.4 ± 11.4	19.6 ± 0.4
	X ray + capsule + Locke's solution	4	8.4 ± 4.3	4.5 ± 1.2	74.1 ± 9.1	16.8 ± 1.1
	X ray + marrow, intraperitoneally	6	48.8 ± 7.1	8.7 ± 1.0	91.3 ± 3.5	18.7 ± 0.2
2	X ray only	5	25.1 ± 2.8	5.7 ± 0.2	76.5 ± 3.5	20.1 ± 0.9
	X ray + capsule + marrow	4	11.2 ± 5.1	5.0 ± 0.8	81.1 ± 8.5	19.0 ± 1.1
	X ray + capsule + Locke's solution	5	13.8 ± 1.4	6.0 ± 0.5	100.1 ± 10.0	18.9 ± 0.3

II. Subcellular Fractionation

In two experiments performed by Dr. Sarah K. Hochstetler, the work of Cole, Fishler, and Bond cited above was repeated and confirmed. In the first, randomized groups of female mice were irradiated by our usual technique. Their male litter mates, used as marrow donors, were about 60 days of age at sacrifice. The marrow from both femurs of each donor was homogenized for 3 minutes in a glass microhomogenizer in 0.5 cc. of the sucrose-salt-ATP medium described by Cole *et al.* (*8*) while immersed in cracked ice. The first centrifugation was carried out at 600 g (1,730 r.p.m.) for 10 minutes at 3° C. and the sediment (fraction 1) washed and recentrifuged twice. The supernatant was then spun at 8,500 g (10,800 r.p.m.) for 10 minutes, and the sediment (fraction 2) washed once. The remaining supernatant was spun at 20,000 g (16,600 r.p.m.) for 90 minutes, and both the sediment and final supernatant saved as fractions 3 and 4, respectively. All fractions were injected intravenously on experimental day 25, promptly after the last irradiation. Microscopic examination revealed that fraction 1 was composed of cell nuclei, contaminated with apparently nondisrupted cells to the extent of about 10 cells per 100 nuclei. The other fractions contained no whole cells and corresponded to the mitochondrial, microsome or submicroscopic particle, and supernatant fraction, respectively, of Schneider (*15*). As indicated in table 2, fraction

TABLE 2.—*Effect of differentially centrifuged bone-marrow cell fractions on lymphoid-tissue recovery in irradiated strain C57BL female mice*

Fraction injected	No. of mice	Thymus (*mg.*)	Nodes (*mg.*)	Spleen (*mg.*)	Body weight (*gm.*)
None	6	25.0 ± 4.5	6.2 ± 0.2	75.9 ± 5.4	19.2 ± 0.6
Nuclei	6	*58.2* ± 5.3	7.2 ± 0.6	66.1 ± 4.2	17.7 ± 0.5
Mitochondria	7	29.6 ± 2.4	6.9 ± 0.3	75.0 ± 4.3	19.7 ± 0.5
Submicroscopic particles	7	22.9 ± 2.2	6.4 ± 0.8	63.9 ± 5.4	18.2 ± 0.6
Supernatant	8	23.7 ± 3.4	6.7 ± 0.3	61.4 ± 3.7	18.2 ± 0.4

1 containing nuclei and some whole cells was highly active in promoting regeneration of the irradiated thymus, whereas the cytoplasmic fractions had no activity.

Attempts to prepare nuclei fractions less contaminated by whole cells led to the use of spleen rather than marrow as the starting tissue, as originally described by Cole *et al.* The spleens of young (7- to 10-day-old) C57BL mice seemed to contain less connective tissue and were easier to homogenize than those of 2-month-old animals. Nuclei fractions from spleens of both age groups were prepared essentially as above. Homogenization of the young spleen cells was essentially complete after 1 to 1.5 minutes, whereas those of the 2-month-old mice required a 2-minute homogenization, repeated once after the first centrifugation, to obtain disruption of about 90 percent of the cells. If homogenization was carried further, as in trials with bone marrow, all of the cells could be opened but the centrifuged fraction 1 became viscid due to breakdown of the nuclei. Those from the young animals contained on the average only 1 or 2 whole cells per 500 to 700 nuclei, whereas those from the older mice were almost as heavily contaminated as the marrow nuclei preparations. In preliminary work, intravenous injection of either the whole spleen homogenates or the nuclei fractions was followed within a few seconds by bizarre convulsions and death, apparently of central nervous system origin. Accordingly all injections were made intraperitoneally on day 25. Each animal received whole homogenate or nuclei from 3 spleens of the older donors, or from 5 of the much smaller spleens of the young donors.

Results are presented in table 3. The nuclei fraction from 7- to 10-day-old mice significantly increased thymic weights over those of irradiated controls, whereas the corresponding fraction from the older donors was inactive, and the whole spleen homogenates had dubious activity.

These experiments are of course open to the same objections as those originally described by Cole and his colleagues. It has been pointed out that the effect obtained with the "nuclei" fraction could conceivably be due to the contaminating whole cells in the preparation. This has seemed unlikely on the basis of earlier experiments (*10*) which seemed to suggest

TABLE 3.—*Effect of intraperitoneal injection of spleen cell nuclei*

Material injected	Age of donors (*days*)	Thymus (*mg.*)	
		Males	Females
None; control		18. 2 ± 2. 1 (9)*	24. 4 ± 2. 4 (8)
Whole homogenate from 3 spleens	55–60	25. 8 ± 2. 8 (6)	26. 0 ± 4. 4 (5)
Spleen cell nuclei from 3 spleens	55–60	21. 2 ± 3. 1 (7)	22. 2 ± 1. 9 (8)
Spleen cell nuclei from 5 spleens	7–10	32. 2 ± 4. 2 (8)	42. 4 ± 3. 2 (6)

*Figure in parentheses = number of mice in group.

that a greater number of intact marrow or spleen cells were required to give a definite response than were present in the amount of nuclei fraction injected. However, more recent experiments to be described below indicate that thymic regeneration can be effected by considerably smaller amounts of marrow than we had previously believed. If one attributes the activity to the contaminating whole cells, it is difficult to explain the lack of effect of the nuclei fraction from 2-month-old donors, where contamination with whole cells was about 500 times as great as in the active nuclei fraction from 7- to 10-day-old mice. Whole cell suspensions from these age groups differ in effectiveness against radiation mortality by a factor of only about five. Nonetheless, these experiments cannot be regarded as providing conclusive evidence that the activity resides in cell-free suspensions of marrow or spleen cell nuclei. They do, however, clearly indicate that the cytoplasmic and supernatant fractions are inactive.

III. Role of the Spleen

Since the effects of thigh-shielding and marrow-injection in preventing radiation death and tumor induction parallel those of spleen-shielding (*6, 11*), the question arose whether the activity of the marrow was mediated by or required the presence of an intact spleen. Six groups of 10 mice each were set up from a randomized pool, with the sexes equally represented. Alternate groups were splenectomized. Two days later irradiation was begun on an 8-day interval basis. The first pair of groups (intact and operated) were irradiated with the left thigh shielded. The other groups were unshielded; one pair received marrow cells intravenously after the fourth X-ray dose, while the last pair received no further treatment.

Splenectomy did not prevent or diminish the regenerative effect of thigh-shielding or marrow-injection on lymphoid-tissue weight (table 4). It is concluded that the marrow factor does not act by way of the spleen. This is consistent with other evidence (*19*) suggesting that, on the contrary, the spleen-shielding phenomenon is essentially unique for the mouse and is based upon the relatively important role of the spleen in

on lymphoid-tissue weights in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Males	Females	Males	Females	Males	Females
10. 6 ± 1. 3 (9)	5. 6 ± 0. 4 (8)	78. 3 ± 4. 0 (9)	79. 3 ± 3. 2 (8)	24. 2 ± 0. 4 (9)	20. 5 ± 0. 4 (8)
5. 0 ± 1. 2 (5)	7. 0 ± 0. 7 (5)	102. 0 ± 16. 3 (6)	76. 2 ± 4. 3 (5)	22. 9 ± 0. 5 (6)	19. 2 ± 0. 8 (5)
7. 9 ± 0. 8 (7)	5. 2 ± 0. 5 (8)	70. 5 ± 4. 3 (7)	75. 0 ± 5. 2 (8)	22. 9 ± 0. 7 (7)	18. 8 ± 0. 4 (8)
12. 8 ± 1. 2 (8)	6. 8 ± 0. 4 (6)	82. 4 ± 5. 1 (7)	79. 5 ± 3. 3 (6)	21. 6 ± 0. 4 (8)	18. 5 ± 0. 6 (6)

this species as an extraskeletal, marrow-like tissue. In two experiments, intravenously injected spleen cell suspensions were compared with marrow cell suspensions in terms of lymphoid-tissue weight response. In the first experiment, mice about 60 days old were used as a source of both tissues. It was found that injection of suspensions more concentrated than 1 spleen per 1.5 ml. of Locke's solution produced convulsions and death. Therefore each spleen was suspended in 2.0 ml. of Locke's solution and each test animal received 0.5 ml. (one-fourth spleen) intravenously. The marrow-injected group received all of the marrow from two femurs in 0.5 ml. of Locke's solution. In the second experiment, the donors were 1-day-old C57BL mice, and two spleens were suspended in 0.5 ml. of Locke's solution. As table 5 reveals, the spleen cell suspensions of the older mice

TABLE 4.—*Effect of splenectomy on activity of the marrow factor in*

Operation	Treatment (other than irradiation)	Thymus (*mg.*)	
		Males	Females
Intact	Thigh-shielded	63.2 ± 4.9 (5)*	77.0 ± 7.10 (4)
Splenectomy	Thigh-shielded	58.1 ± 1.3 (5)	78.1 ± 4.3 (5)
Intact	Marrow, intravenously	58.4 ± 3.7 (5)	70.1 ± 5.8 (5)
Splenectomy	Marrow, intravenously	51.2 ± 3.3 (5)	63.1 ± 1.7 (5)
Intact	None	20.6 ± 3.7 (5)	18.9 ± 2.6 (5)
Splenectomy	None	16.6 ± 1.8 (4)	21.3 ± 1.9 (6)

*Figure in parentheses=number of mice in group.

TABLE 5.—*Effect of intravenous injection of newborn and adult spleen cell*

Material injected	Age of donor (*days*)	Thymus (*mg.*)	
		Males	Females
Locke's solution	———	18. 9 ± 2. 2 (5)*	35. 9 ± 8. 1 (5)
Bone marrow	60	62. 4 ± 5. 0 (5)	73. 0 ± 5. 9 (5)
Spleen	60	35. 3 ± 3. 2 (10)	43. 7 ± 9. 5 (5)
Locke's solution	———	22. 0 ± 2. 0 (5)	49. 2 ± 8. 3 (4)
Spleen	1	44. 4 ± 5. 1 (5)	69. 9 ± 8. 1 (5)

*Figure in parentheses=number of mice in group.

were only about 60 percent as active as the bone-marrow cell suspensions, in the amounts injected. The spleens of 1-day-old mice were almost as active as adult bone marrow, however. In addition to age, genetic constitution may also influence relative activity of the spleen (*19*), to a degree which is roughly proportional to the extramedullary hematopoietic function of the spleen of each strain.

IV. Modification of Marrow or Spleen Activity by Pretreatment of Donor Mice

A. *Phenylhydrazine and turpentine.*—In an attempt to discover which cell type contained the active principle, an intense hyperplasia of the erythroid or myeloid series in both marrow and spleen was induced by treat-

promoting lymphoid-tissue recovery in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Males	Females	Males	Females	Males	Females
13.8 ± 1.9 (5)	16.1 ± 3.4 (4)	83.1 ± 3.1 (5)	90.5 ± 8.5 (4)	23.4 ± 0.9 (5)	20.4 ± 1.0 (4)
17.0 ± 2.0 (5)	14.9 ± 1.3 (5)	———	———	23.1 ± 0.6 (5)	20.1 ± 0.8 (5)
9.8 ± 0.4 (5)	12.6 ± 0.9 (5)	74.4 ± 3.3 (5)	85.0 ± 8.0 (5)	23.2 ± 0.3 (5)	20.2 ± 0.5 (5)
16.5 ± 1.0 (5)	13.3 ± 2.9 (5)	———	———	25.4 ± 0.6 (5)	20.7 ± 0.8 (5)
4.6 ± 0.4 (5)	5.9 ± 0.8 (5)	67.3 ± 4.9 (5)	78.6 ± 6.4 (5)	23.2 ± 0.4 (5)	20.1 ± 0.5 (5)
6.5 ± 1.1 (4)	7.4 ± 1.0 (6)	———	———	23.2 ± 1.0 (4)	19.2 ± 0.4 (6)

suspensions on lymphoid-tissue weights in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Males	Females	Males	Females	Males	Females
8. 2 ± 0. 4 (5)	7. 2 ± 0. 8 (5)	74. 7 ± 3. 2 (5)	67. 9 ± 3. 3 (5)	22. 9 ± 0. 3 (5)	19. 5 ± 0. 6 (5)
13. 5 ± 1. 2 (5)	15. 5 ± 0. 6 (5)	78. 7 ± 3. 6 (5)	92. 4 ± 3. 2 (5)	23. 0 ± 0. 8 (5)	19. 2 ± 0. 6 (5)
10. 1 ± 1. 4 (10)	9. 3 ± 0. 5 (5)	77. 2 ± 3. 8 (10)	85. 9 ± 3. 4 (5)	21. 2 ± 0. 5 (10)	18. 8 ± 0. 8 (5)
5. 0 ± 0. 6 (5)	10. 2 ± 2. 4 (4)	61. 6 ± 3. 3 (5)	70. 6 ± 5. 6 (4)	21. 2 ± 0. 6 (5)	18. 2 ± 0. 8 (4)
15. 4 ± 2. 7 (5)	11. 2 ± 2. 3 (5)	81. 8 ± 3. 0 (5)	87. 1 ± 3. 4 (5)	21. 0 ± 0. 6 (5)	17. 9 ± 0. 3 (5)

ment of donor mice with phenylhydrazine and turpentine, respectively. The dose of phenylhydrazine was 1.25 mg. per day for 2 days, the animals being sacrificed 2 days later; a single 0.1-ml. subcutaneous injection of turpentine was used, and the mice were killed 4 days later. Cell suspensions were prepared in the usual way and injected intravenously. Marrow from untreated donors of the same age was injected in equal amount into one control group, while another received only Locke's solution after irradiation. An extra group received normal marrow intramuscularly.

Microscopic examination revealed an intense hyperplasia of and overgrowth by erythroid cells in the phenylhydrazine-treated marrows. At 2 days after turpentine injection, the marrow was densely packed with myeloblasts and some mature polymorphonuclear leukocytes; the latter cell greatly predominated at 4 days. Neither type of alteration of marrow-cell population appeared to affect marrow activity, however (table 6). In another experiment, which was performed by Dr. William Faraghan, the spleens of phenylhydrazine-treated donors similarly exhibited the same activity as those of normal donors. This result suggests that some more primitive cell type in the marrow (and spleen) may contain the active factor, but direct evidence for this view is not yet at hand.

B. *Sex hormones.*—Testosterone inhibits the development of thymic lymphosarcomas in irradiated C57BL mice, whereas estrogens are lymphomogenic (*20*, *21*). It seemed possible that these hormones might affect the bone-marrow factor in opposite ways, and thus account at least in part for the difference in their effect on tumorigenesis. Two experiments have been performed in which donor mice have been injected with one or the other hormone for 2½ weeks, sacrificed, and their marrows injected into irradiated test animals, with controls receiving untreated marrow for comparison. The results of both experiments were similar

TABLE 6.—*Effect of induced hyperplasia of erythroid*

Treatment of donor mice	Material injected and route of administration	Thymus (*mg.*)	
		Male	Female
Turpentine	Marrow, intravenously	59. 2 ± 3. 9 (6)*	74. 1 ± 9. 7 (5)
Phenylhydrazine	Marrow, intravenously	58. 6 ± 3. 9 (6)	56. 5 ± 6. 8 (6)
Control	Marrow, intravenously	68. 2 ± 4. 7 (6)	78. 4 ± 3. 0 (5)
Control	Marrow, intramuscularly	24. 5 ± 2. 4 (5)	32. 7 ± 8. 5 (5)
Control	Placebo, intravenously	29. 3 ± 4. 1 (6)	29. 9 ± 4. 0 (6)

*Figure in parentheses=number of mice in group.

and are partly consolidated in table 7; there was no significant inhibition or stimulation of the marrow factor by either hormone.

V. Preservation and Storage of Marrow

Marrow from 60-day-old C57BL donors was divided into two portions. One was frozen gradually, by successive cooling to 3° C. over crushed ice, next to about −10° C., and finally stored at −70° C. The other portion was quick-frozen directly over dry ice and stored at −70° C. Both were kept at this temperature for 28 days. The first fraction was thawed slowly by a reversal of procedure; the second by removal from the cold directly into a 37° C. water bath. They were then suspended in 0.5 ml. of Locke's solution and injected intravenously into irradiated test animals, with 2 groups of controls receiving fresh normal marrow and Locke's solution, respectively.

In another experiment, conducted by Dr. William Faraghan, freshly removed marrow was frozen in acetone-dry ice and lyophilized. The frozen-dried material was then resuspended in cold Locke's solution for intravenous injection, with controls as above. The results of both experiments, summarized in table 8, indicate that activity of the marrow factor was destroyed by freezing and lyophilization.

VI. Dose of Marrow Versus *Effect*

In an earlier study (*10*), marrow from both femurs of a single donor was effective when injected intravenously into a single irradiated recipient ("1:1" dose), whereas there was little evidence of activity when the marrows of 2 donors were divided among 5 recipients. This and other evidence suggested that relatively large amounts of marrow were required. This question was restudied in two experiments in which graded dilutions of normal marrow in Locke's solution were injected intravenously into

or granulocytic series on activity of marrow

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female	Male	Female
14.5 ± 1.1 (6)	10.2 ± 1.8 (5)	84.4 ± 3.6 (6)	82.8 ± 3.0 (5)	21.7 ± 0.9 (6)	17.8 ± 0.5 (5)
15.9 ± 1.5 (6)	16.6 ± 2.1 (6)	81.7 ± 5.3 (6)	81.8 ± 5.1 (6)	21.1 ± 0.2 (6)	18.5 ± 0.5 (6)
13.7 ± 2.2 (6)	12.1 ± 0.8 (5)	83.7 ± 6.6 (6)	87.0 ± 6.6 (5)	22.5 ± 0.6 (6)	19.1 ± 1.0 (5)
9.9 ± 2.8 (5)	10.6 ± 2.8 (5)	64.8 ± 2.8 (5)	84.5 ± 5.1 (5)	21.3 ± 0.9 (5)	18.1 ± 0.4 (5)
7.8 ± 1.3 (6)	9.0 ± 0.7 (6)	65.6 ± 3.4 (6)	70.1 ± 3.8 (6)	22.5 ± 0.7 (6)	18.5 ± 0.5 (6)

TABLE 7.—*Effect of sex hormone pretreatment of donor mice*

Treatment of donor mice	Material and route	Thymus (*mg.*)	
		Male	Female
Testosterone*.....	Marrow, intravenously.....	63.0 ± 4.0 (6)†	73.1 ± 3.7 (6)
Testosterone.....	Marrow, intravenously.....	29.9‡ ± 5.9 (5)	59.8 ± 15.9 (4)
Estrogen§.....	Marrow, intravenously.....	53.2 ± 3.7 (5)	73.4 ± 9.4 (4)
Estrogen.....	Marrow, intravenously.....	47.8 ± 6.7 (4)	69.8 ± 8.6 (4)
Control.....	Marrow, intravenously.....	54.9 ± 3.9 (5)	65.6 ± 7.9 (5)
Control.....	Locke's solution, intravenously....	29.3 ± 4.1 (6)	25.0 ± 4.0 (6)

*Testosterone propionate (Schering) aqueous, 0.5. mg. 3 times weekly; 11 injections.
†Figure in parentheses=number of mice in group.

TABLE 8.—*Effect of freezing or lyophilization on activity*

Material injected	Amount injected	Thymus (*mg.*)	
		Male	Female
"Slow-frozen" marrow.....	contents of 4 femurs.	21.8 ± 1.0 (5)*	32.2 ± 6.1 (5)
"Quick-frozen" marrow.....	contents of 4 femurs.	31.9 ± 1.9 (5)	32.3 ± 5.4 (5)
Normal marrow.....	contents of 2 femurs.	63.3 ± 3.5 (5)	77.7 ± 4.3 (5)
Locke's solution.....	0.5 ml.....	22.0 ± 2.0 (5)	49.2 ± 8.3 (4)
Locke's solution.....	0.5 ml.....	26.2 ± 5.0 (5)	24.8 ± 3.4 (5)
Normal marrow.....	contents of 2 femurs.	56.1 ± 3.9 (5)	72.4 ± 2.9 (5)
Lyophilized marrow.....	contents of 4 femurs.	18.0 ± 1.4 (10)	28.2 ± 2.0 (10)

*Figure in parentheses=number of mice in group.

test animals in a constant volume of 0.5 ml. In this first experiment, this volume contained the entire marrow from 1 to 4 mouse femurs, and all were about equally (and maximally) effective. Accordingly, another experiment was performed in which the effective amount injected was that derived from 2, 1, ½, ¼, ⅛, or 1/16 femoral marrow content, respectively. An aliquot of these marrow cell suspensions was stained with 0.02-percent gentian violet in 3 percent acetic acid and counted in a hemacytometer,

on activity of injected marrow in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female	Male	Female
15.0 ± 0.8 (5)	13.0 ± 1.4 (6)	78.0 ± 3.3 (5)	89.9 ± 6.3 (6)	20.8 ± 0.7 (6)	18.2 ± 1.0 (6)
11.2 ± 1.4 (5)	11.4 ± 2.3 (4)	73.5 ± 1.7 (5)	87.0 ± 8.5 (4)	23.5 ± 0.5 (5)	19.8 ± 0.5 (4)
12.0 ± 1.5 (5)	12.5 ± 1.8 (4)	87.2 ± 6.2 (5)	99.4 ± 5.1 (4)	22.4 ± 0.9 (5)	18.6 ± 0.8 (4)
9.2 ± 0.9 (4)	10.1 ± 1.6 (4)	80.1 ± 4.7 (4)	77.6 ± 3.8 (4)	23.8 ± 0.8 (4)	19.3 ± 0.7 (4)
10.8 ± 1.5 (5)	11.3 ± 1.2 (5)	71.0 ± 2.8 (4)	89.7 ± 5.1 (5)	21.4 ± 0.5 (5)	19.5 ± 0.7 (5)
7.8 ± 1.3 (6)	9.0 ± 0.4 (6)	65.6 ± 3.4 (6)	70.1 ± 3.8 (6)	22.5 ± 0.7 (6)	18.5 ± 0.5 (6)

‡Some intravenous marrow injections were unsatisfactory.
§Dienestrol (White) aqueous, 25.0 mg. 3 times weekly; 11 injections; killed 24 hours after last injection.

of the marrow factor in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female	Male	Female
8.9 ± 1.4 (5)	8.4 ± 1.2 (5)	59.1 ± 2.9 (5)	82.8 ± 9.8 (5)	20.9 ± 0.4 (5)	19.2 ± 0.8 (5)
8.5 ± 0.9 (5)	8.0 ± 1.4 (5)	67.5 ± 2.5 (5)	74.4 ± 5.1 (5)	21.8 ± 0.4 (5)	17.4 ± 0.4 (5)
14.7 ± 1.9 (5)	12.4 ± 2.4 (5)	85.7 ± 2.9 (5)	89.7 ± 2.1 (5)	22.5 ± 0.5 (5)	18.8 ± 0.5 (5)
5.0 ± 0.6 (5)	10.2 ± 2.4 (4)	61.6 ± 3.3 (5)	70.6 ± 5.6 (4)	21.2 ± 0.6 (5)	18.2 ± 0.8 (4)
9.6 ± 1.2 (5)	10.5 ± 1.4 (5)	60.1 ± 1.3 (5)	80.1 ± 3.6 (5)	21.0 ± 0.8 (5)	20.5 ± 0.4 (5)
9.8 ± 1.1 (5)	15.9 ± 2.5 (5)	80.5 ± 2.9 (5)	102.0 ± 3.6 (5)	22.5 ± 0.5 (5)	20.3 ± 0.7 (5)
10.6 ± 1.2 (10)	9.6 ± 1.1 (10)	66.3 ± 2.6 (10)	78.7 ± 5.4 (10)	23.1 ± 0.5 (10)	19.7 ± 0.7 (10)

yielding the following cell counts per cubic millimeter, in the same sequence: 43,475; 21,275; 9,862; 3,700; 2,138; and 1,012. Two groups of recipients were used at the 2-femur dose level: one received the marrow from both femurs of individual donors as soon as it could be suspended, whereas the other received the same amount from the pooled marrows of 15 donors. The donors and recipients were of the same sex. A control group received Locke's solution alone. The data are presented in table 9.

TABLE 9.—*Relation between amount of bone marrow injected and*

Expt. No.	Material injected	Amount injected	Thymus (*mg.*)	
			Male	Female
1	Locke's solution	0.5 ml	20.4 ± 4.4 (5)*	26.9 ± 3.0 (6)
	Marrow	4 femurs	73.9 ± 3.2 (5)	88.9 ± 4.3 (5)
	Marrow	2 femurs	65.4 ± 3.7 (5)	79.0 ± 4.0 (5)
	Marrow	1 femur	64.6 ± 4.8 (7)	76.3 ± 2.8 (7)
2	Locke's solution	0.5 ml	23.4 ± 4.0 (5)	22.9 ± 2.4 (5)
	Marrow	2 femurs (individual)	74.8 ± 6.6 (5)	77.1 ± 3.0 (4)
	Marrow	2 femurs (pooled)	62.8 ± 3.7 (5)	82.8 ± 3.6 (5)
	Marrow	1 femur	64.8 ± 5.0 (5)	74.9 ± 1.8 (6)
	Marrow	one-half femur	57.7 ± 6.8 (5)	75.3 ± 5.0 (5)
	Marrow	one-fourth femur	46.7 ± 4.3 (5)	74.7 ± 1.6 (3)
	Marrow	one-eighth femur	45.6 ± 3.5 (5)	65.5 ± 4.4 (5)
	Marrow	one-sixteenth femur	46.0 ± 6.7 (4)	51.1 ± 4.8 (5)

*Figure in parentheses=number of mice in group.

In computing[5] the regression of thymic weight on quantity of marrow injected, the 2-femur aliquot from pooled marrow and the 2-femur group from individual donors were combined, since the difference in average thymic weight was for neither sex large enough to be statistically significant, and in addition, the differences observed were of opposite size, further indicating their random nature. A straight-line regression of thymic weight on logarithm of marrow dose was computed separately for each sex. In each case a test of linearity was carried out and the values of the F statistic lay below the 50 percent point for males and below the upper 10 percent for females, indicating that the assumption of a straight-line regression on log dose is not unreasonable. The slopes of the two lines were nearly the same, differing by about two-thirds of the standard error of their difference. Accordingly, two lines having a common slope (a weighted average of the slopes for the two sexes) were fitted to the data

[5] It is indeed a pleasure to acknowledge our indebtedness to Dr. Lincoln E. Moses, Assistant Professor of Statistics, Stanford University, for his assistance in the planning of several of the experiments in this paper, and in particular for his statistical analysis of this experiment, which is quoted almost verbatim here.

lymphoid-tissue weight response in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female	Male	Female
6.7 ± 0.9 (5)	6.4 ± 0.3 (6)	61.5 ± 4.2 (5)	70.7 ± 4.7 (6)	22.6 ± 0.7 (5)	18.8 ± 0.4 (6)
9.2 ± 0.6 (5)	11.1 ± 1.1 (5)	67.1 ± 3.2 (5)	108.4 ± 5.5 (5)	21.6 ± 0.6 (5)	20.3 ± 0.7 (5)
12.0 ± 1.2 (5)	12.8 ± 1.6 (5)	72.4 ± 3.3 (5)	91.8 ± 6.3 (5)	23.0 ± 0.4 (5)	19.1 ± 0.4 (5)
10.0 ± 0.8 (7)	10.0 ± 0.6 (7)	75.0 ± 4.6 (6)	78.5 ± 3.1 (7)	22.2 ± 0.6 (7)	18.4 ± 0.5 (7)
5.1 ± 0.4 (5)	7.1 ± 0.8 (5)	65.0 ± 1.3 (5)	78.5 ± 11.2 (5)	20.8 ± 0.4 (5)	18.4 ± 0.6 (5)
11.1 ± 1.3 (5)	11.8 ± 2.1 (4)	76.0 ± 2.6 (5)	81.4 ± 6.8 (4)	21.6 ± 0.6 (5)	18.1 ± 1.0 (4)
9.2 ± 0.8 (5)	13.6 ± 3.0 (5)	75.3 ± 5.5 (5)	87.1 ± 5.2 (5)	21.0 ± 0.7 (5)	19.0 ± 0.5 (5)
8.1 ± 0.6 (5)	12.9 ± 1.4 (6)	71.2 ± 3.0 (5)	79.8 ± 2.0 (6)	21.0 ± 0.3 (5)	18.6 ± 0.2 (6)
10.1 ± 1.2 (5)	11.2 ± 2.9 (5)	68.2 ± 3.7 (5)	80.6 ± 5.0 (5)	21.2 ± 0.7 (5)	18.4 ± 0.5 (5)
9.8 ± 2.3 (5)	9.7 ± 0.2 (4)	77.3 ± 5.5 (5)	94.2 ± 2.8 (4)	21.4 ± 0.9 (5)	20.4 ± 0.4 (4)
7.6 ± 0.3 (5)	10.5 ± 2.6 (5)	75.2 ± 7.4 (5)	79.7 ± 3.4 (5)	21.1 ± 0.8 (5)	19.0 ± 0.9 (5)
6.1 ± 0.9 (4)	6.6 ± 0.8 (5)	66.1 ± 1.3 (4)	75.2 ± 4.0 (5)	22.0 ± 0.5 (4)	18.3 ± 0.3 (5)

of the two sexes (text-fig. 1). It can be seen from the figure (or from the equation below) that doubling the dose of marrow injected (over the range of doses employed) increased the average thymic weight at experimental day 50 by about 5.39 ± 1.27 mg. The two regression equations are:

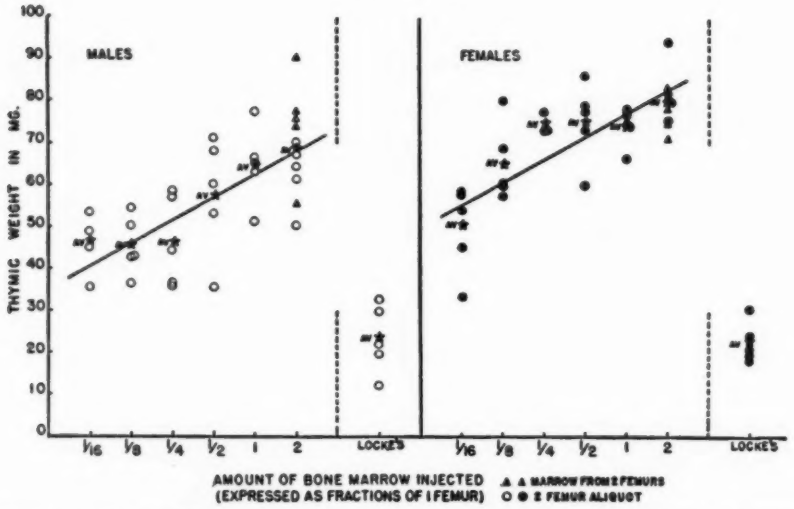
$$\text{Females}—\tilde{y}=55.7 \pm 5.39 \log_2 (16F)$$

$$\text{Males}—\tilde{y}=41.0 \pm 5.39 \log_2 (16F)$$

where $\tilde{y}$ is the estimated average thymic weight to be found at dose F and $\log_2 (16F)$ is that power of Z which is equal to 16 times the aliquot F. Thus an aliquot F = ¼ corresponds to $\log_2 (16F) = \log_2 (4) = 2$.

VII. Distribution of P^{32}-labeled Bone Marrow after Intravenous Injection

Although it is evident that the marrow factor promotes regeneration of the radiation-injured thymus and lymph nodes, there has been little information regarding the distribution and fate of the intravenously injected marrow cell suspensions. Congdon, Uphoff, and Lorenz (*5*)



TEXT-FIGURE 1.—Effect of injections of varying amounts of bone marrow on thymic weights of irradiated C57BL mice.

described the histologic sequence of events in intraperitoneal marrow implants, and noted focal nodular lesions in the lungs of intravenously injected animals which represented the sole microscopic evidence of localization of the marrow.

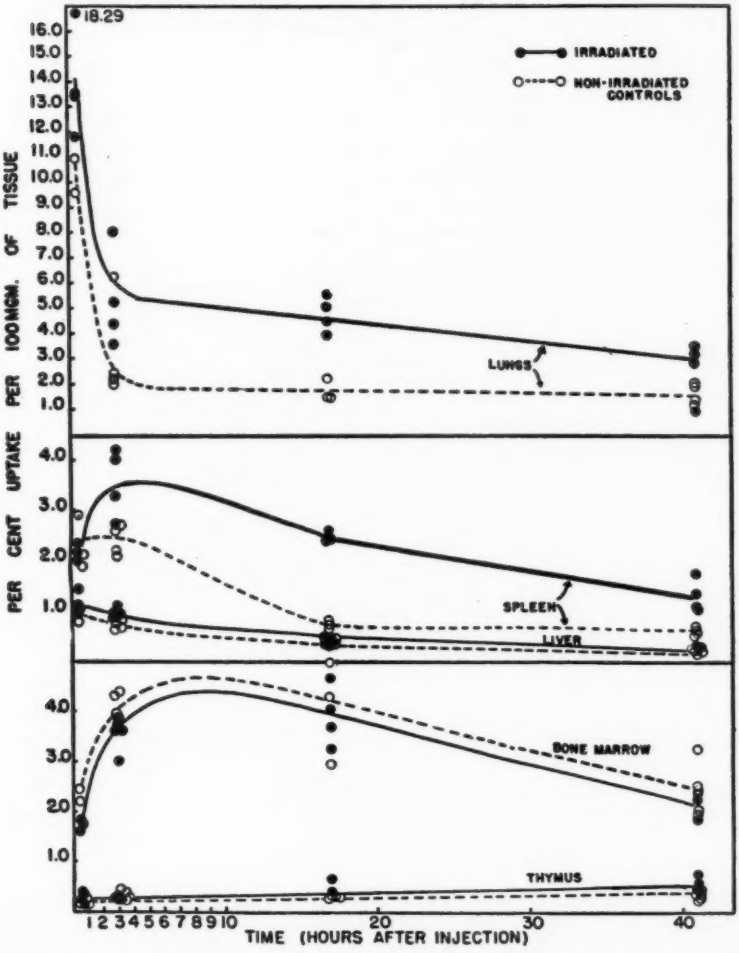
The experiments to be described, which were performed by Dr. Paul Toch, suffer from the inherent difficulty that there is no certainty that the isotopic label is distributed after injection in the same way as the unidentified active factor in the marrow. Nonetheless, these studies were undertaken as a first approach to the problem.

Marrow obtained from the femora of donor mice was pooled, suspended in Locke's solution and incubated with carrier free P^{32} phosphate for 2 hours at 37° C. under gentle agitation. After completion of the incubation the suspension was centrifuged at 2,000 r.p.m. for 10 minutes, the supernatant was pipetted off and the marrow cells were resuspended in Locke's solution. This procedure was repeated three times or until the ratio of counts per unit volume of cells *versus* supernatant was at least ten to one. An aliquot of 0.4 cc. of the final suspension was injected into a tail vein of each experimental animal. Groups of mice were then sacrificed at appropriate intervals. Blood, bone marrow, thymus, lungs, liver, and spleen were weighed wet, transferred to test tubes and treated by wet ashing with 15 percent KOH. Aliquots were then plated and their radioactivity determined.

In a preliminary experiment, 8 nonirradiated 60 ± 3-day-old male C57BL mice were used to test the feasibility of the procedure. Four of these were sacrificed between 17 and 32 minutes after injection of the bone-marrow suspension and two each were sacrificed after 1.5 and 4.5 hours, respectively.

In the second experiment 32 mice, 30 ± 3 days old, were used—divided equally between the sexes. Sixteen of these were irradiated with 168 r × 4 every 8 days; the remainder served as nonirradiated controls. Both irradiated and nonirradiated animals were randomly distributed into four groups to be sacrificed 0.5, 3, 17, and 41 hours after injection of the tagged bone marrow. All mice were injected on the day of the last X-ray treatment. Blood sampling was omitted in this experiment and bone marrow was transferred directly to copper counting cups without wet ashing and allowed to dry. Results for bone marrow are therefore expressed on a dry weight basis. Other tissues were treated as described above.

The results are summarized in tables 10 and 11 and in text-figure 2.



TEXT-FIGURE 2.—Percent uptake per 100 mg. of tissue of intravenously injected P^{32}-labeled bone-marrow suspension.

TABLE 10.—*Percent uptake per 100 mg. of tissue of intravenously injected P^{32}-labeled bone-marrow suspension*

Time (*minutes*)	Blood	Marrow	Thymus	Lungs	Liver	Spleen
17	0. 24	3. 07	0. 31	11. 56	1. 10	1. 24
23	0. 33	1. 67	0. 38	17. 60	1. 06	1. 63
27	0. 22	7. 21	0. 29	21. 64	1. 08	2. 74
32	0. 08	1. 90	0. 18	9. 78	0. 87	2. 96
88	0. 13	1. 85	0. 15	7. 77	0. 74	3. 58
97	0. 14	2. 13	0. 20	9. 63	1. 08	3. 02
275	0. 05	1. 36	0. 44	10. 80	0. 62	2. 81
278	0. 06	1. 79	0. 19	6. 38	0. 69	2. 39

The lungs showed the greatest uptake of injected tagged bone marrow (18%). This occurred rapidly (by 20 to 30 minutes), but the activity was also lost fairly rapidly. Controls take up somewhat less than irradiated animals (15%), and lose activity to a greater extent (text-fig. 2). The livers of both irradiated and nonirradiated animals took up approximately 10 percent of the activity of the tagged bone marrow and lost activity more slowly than the lungs. Concentration of radioactivity per 100 mg. of liver tissue was rather low (1%); thus, the relatively high uptake by the total organ is due to its large size. The marrow and spleen reached peak concentrations of radioactivity somewhat later than the lungs and liver (after 90 minutes). The spleens of irradiated animals showed greater concentrations of radioactivity than those of nonirradiated controls; however, uptakes per total organ were comparable or somewhat lower in the irradiated mice. This difference may be accounted for by the smaller spleen size of the irradiated as compared with nonirradiated mice. Only traces of radioactivity were found in blood (see table 10) and thymus.

It is concluded that intravenously injected bone marrow is taken up primarily by the reticuloendothelial system; however, about 70 percent of the injected radioactive material is not accounted for in the organs examined. The lungs and liver take up the bulk of the injected material, the highest concentrations being found in the former. The lungs of irradiated animals take up somewhat more radioactivity than those of nonirradiated controls; the reason for this is not clear. Only traces of radioactivity are found in blood and thymus.

VIII. Screening for Activity in Other Tissues, Strains, and Species

A. *Adult homologous tissues.*—Various organs were excised from C57BL mice, minced and ground in glass microhomogenizers in cold Locke's solution at 3° C. until they passed readily through a #20-gauge needle, and were injected intraperitoneally into irradiated C57BL test mice. The intraperitoneal route was used after preliminary work indicated an excessive mortality, due to pulmonary embolism or cerebral thrombosis, following intravenous injection of suspensions of the same tissues. Controls received normal homologous bone marrow intravenously or Locke's

TABLE 11.—*Percent uptake per 100 mg. of tissue of intravenously injected P^{32}-labeled bone-marrow cell suspensions*

Time	Marrow *		Thymus		Lungs		Liver		Spleen	
	X ray	Control	X ray	Control	X ray	Control	X ray	Control	X ray	Control
20 to 30 minutes	1. 61	2. 23	0. 23	0. 10	11. 79	9. 60	0. 97	0. 78	2. 33	1. 92
	1. 82	2. 45	0. 39	0. 19	18. 29	10. 95	1. 42	1. 08	2. 17	2. 91
	0. 94	†	0. 12	†	13. 47	†	1. 05	†	1. 98	†
	1. 79	†	0. 36	†	13. 54	†	1. 12	†	2. 15	†
Average	1. 54	2. 34	0. 28	0. 15	14. 27	10. 28	1. 14	0. 93	2. 16	2. 42
3 hours	3. 94	3. 65	0. 33	0. 29	3. 58	2. 44	0. 96	0. 72	4. 21	2. 16
	3. 64	3. 87	0. 27	0. 33	5. 42	2. 25	0. 86	0. 64	4. 02	2. 20
	3. 73	4. 34	0. 25	0. 29	4. 35	2. 00	0. 95	0. 84	2. 74	2. 71
	3. 01	4. 37	0. 28	0. 33	8. 30	6. 26	1. 12	0. 79	3. 30	2. 65
Average	3. 58	4. 06	0. 28	0. 31	5. 41	3. 24	0. 97	0. 75	3. 57	2. 43
17 hours	3. 26	2. 96	0. 63	0. 26	3. 97	1. 45	0. 46	0. 29	2. 61	0. 66
	4. 03	4. 29	0. 30	0. 32	5. 08	2. 20	0. 42	0. 30	2. 49	0. 76
	3. 68	4. 98	0. 37	0. 31	4. 53	1. 44	0. 48	0. 33	2. 48	0. 80
	4. 67	†	0. 40	†	5. 58	†	0. 50	†	2. 42	†
Average	3. 91	4. 08	0. 43	0. 30	4. 79	1. 70	0. 47	0. 31	2. 50	0. 74
41 hours	1. 97	2. 08	0. 45	0. 25	3. 21	2. 09	0. 18	0. 17	1. 08	0. 59
	2. 38	2. 43	0. 44	0. 32	0. 97	1. 20	0. 21	0. 21	1. 06	0. 55
	1. 88	2. 52	0. 58	0. 39	2. 87	1. 41	0. 31	0. 25	1. 36	0. 65
	2. 25	3. 26	0. 75	0. 34	3. 55	2. 01	0. 30	0. 27	1. 75	0. 70
Average	2. 12	2. 57	0. 56	0. 33	2. 65	1. 68	0. 25	0. 23	1. 31	0. 62

*Activity of bone marrow expressed as percent of injected material per 100 mg. of dry weight.
†Unsatisfactory intravenous injection.

solution intraperitoneally. Although no intraperitoneal marrow control was used here, other evidence (table 1) indicates that activity by this route is readily demonstrable, though appreciably less than that following intravenous injection.

Results are seen in table 12. It is apparent that, in the relatively large amounts injected, none of the tissues tested gave evidence of activity. Infection was prominent in the group injected with small intestine, accounting for the high spleen weights noted. In another experiment, one group of test animals received intravenous 0.5-ml. injections of homologous whole mouse blood weekly for 3 weeks beginning after the last irradiation. A second group received twice weekly 0.2-ml. subcutaneous injections of human blood serum for 2 weeks after irradiation. These treatments had no effect on lymphoid tissue weights (table 12).

B. *Fetal tissues.*—Jacobson (*3*) has reported that minced mouse embryos significantly reduced mortality after total-body irradiation. Homogenates of whole fetuses, and of several fetal organs individually, were made from 15- to 18-day C57BL fetuses by grinding with Locke's solution in a glass microhomogenizer. Preliminary studies revealed that fetal

TABLE 12.—*Effect of various tissue-cell suspensions on*

Expt. No.	Material injected	Amount injected	Thymus (*mg.*)	
			Male	Female
1	Liver	1 lobe in 1.0 ml	20.6 ± 1.71 (6)*	25.4 ± 4.6 (6)
	Kidney	1 kidney in 1.0 ml	23.0 ± 2.6 (6)	23.6 ± 3.4 (6)
	Lymph nodes	4 in 0.5 ml	20.7 ± 2.5 (6)	22.8 ± 3.0 (5)
	Brain	whole brain in 1.0 ml	16.5 ± 3.1 (3)	29.9 ± 6.8 (3)
	Thymus	whole thymus in 0.5 ml	20.9 ± 1.3 (6)	23.8 ± 2.6 (6)
	Intestine	3 cm. in 0.5 ml	14.7 ± 4.2 (5)	23.2 ± 6.0 (4)
	Marrow	2 femurs‡ in 0.5 ml	62.1 ± 2.3 (5)	68.9 ± 3.7 (6)
	Locke's solution	0.5 ml. I. P.	18.8 ± 1.8 (6)	24.1 ± 6.5 (4)
2	Control			25.1 ± 2.8 (5)
	Whole blood			16.3 ± 3.6 (6)
	Serum			24.9 ± 2.5 (8)

*Figure in parentheses = number of mice in group.
†This group became obviously infected by organisms in the injected intestinal material.

lung homogenates were highly lethal, apparently as a result of cerebral thrombosis, upon intravenous injection. Accordingly, a group originally designated to receive lung tissue was added to the group injected with fetal liver. All injections were made intravenously in the following concentrations: 1 whole fetus in 50.0 ml. of Locke's solution, 1 whole liver in 4.0 ml., 2 spleens in 1.0 ml., and 1 thymus and kidney each in 1 ml. The injection volume was 0.5 ml. for all tissues. It was noted that the fetal livers filled almost the entire abdominal cavity at all gestational ages encountered. The spleen was a thin, whitish structure until about 2 or 3 days before the end of gestation, when it became red, suggesting that the spleen does not play an important part in hematopoiesis until late in the gestation period.

The results are summarized in table 13. Fetal liver was moderately effective in promoting thymic weight recovery, whereas whole fetus and the other fetal tissues were inactive under these experimental conditions.

C. *Heterologous marrow.*—Lorenz and his associates (*4*, *7*) found some protective activity of heterologous marrow and bone in irradiated mice, using survival as their criterion. These observations have been thought

lymphoid-tissue recovery in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female	Male	Female
7. 5 ± 1. 2 (6)	9. 9 ± 1. 6 (6)	65. 4 ± 1. 6 (6)	73. 8 ± 4. 3 (6)	21. 7 ± 0. 5 (6)	18. 8 ± 0. 2 (6)
7. 4 ± 0. 8 (6)	12. 0 ± 0. 9 (6)	77. 9 ± 7. 4 (6)	77. 4 ± 5. 6 (6)	22. 5 ± 0. 7 (6)	18. 2 ± 0. 7 (6)
6. 6 ± 0. 3 (6)	8. 9 ± 1. 2 (5)	67. 7 ± 4. 8 (6)	80. 1 ± 3. 2 (5)	21. 4 ± 0. 7 (6)	18. 7 ± 0. 6 (5)
7. 7 ± 2. 0 (3)	7. 8 ± 2. 3 (3)	70. 1 ± 6. 8 (3)	63. 9 ± 8. 8 (3)	21. 6 ± 0. 2 (3)	16. 9 ± 0. 9 (3)
8. 3 ± 0. 8 (6)	11. 2 ± 1. 4 (6)	71. 8 ± 2. 2 (6)	79. 4 ± 6. 3 (6)	23. 8 ± 1. 2 (6)	18. 0 ± 0. 6 (6)
7. 3 ± 0. 9 (5)	8. 4 ± 0. 7 (4)	142.6† ± 15.6 (5)	144.1 ± 17. 9 (4)	19. 8 ± 1. 2 (5)	18. 1 ± 1. 3 (4)
10. 6 ± 1. 8 (5)	15. 5 ± 1. 8 (6)	79. 5 ± 6. 2 (5)	87. 8 ± 5. 9 (6)	22. 2 ± 0. 5 (5)	18. 2 ± 0. 6 (6)
13. 1 ± 1. 6 (6)	8. 9 ± 1. 3 (4)	70. 8 ± 3. 3 (6)	65. 8 ± 8. 7 (3)	22. 4 ± 0. 7 (6)	17. 4 ± 0. 8 (4)
.....	5. 7 ± 0. 2 (5)	.....	76. 5 ± 3. 5 (5)	.....	20. 1 ± 0. 9 (5)
.....	9. 2 ± 1. 2 (6)	.....	64. 6 ± 4. 6 (6)	.....	18. 3 ± 0. 4 (6)
.....	11. 7 ± 0. 8 (8)	.....	85. 0 ± 4. 3 (8)	.....	18. 7 ± 0. 5 (8)

‡Intravenous injection.

TABLE 13.—*Effect of various fetal-tissue homogenates*

Treatment	Thymus (*mg.*)		Nodes (*mg.*)	
	Male	Female	Male	Female
Control; no X ray.....	57.6 ± 1.2 (5)*	60.1 ± 1.9 (5)	15.4 ± 1.6 (5)	17.9 ± 1.3 (5)
X ray only.....	18.4 ± 1.6 (4)	23.0 ± 2.5 (4)	6.8 ± 1.0 (4)	4.9 ± 0.5 (4)
Whole fetal.....	23.8 ± 1.8 (10)	26.4 ± 3.4 (8)	6.6 ± 0.4 (10)	6.6 ± 0.3 (8)
Spleen homogenate....	22.8 ± 2.3 (4)	17.6 ± 5.2 (3)	5.9 ± 0.8 (4)	4.6 ± 1.1 (3)
Liver homogenate.....	38.7 ± 5.0 (9)	34.1 ± 7.4 (8)	8.5 ± 0.6 (9)	7.1 ± 1.0 (8)
Thymus homogenate..	19.4 ± 3.2 (4)	20.1 ± 2.1 (5)	5.3 ± 0.7 (4)	8.6 ± 1.4 (5)
Kidney homogenate...	26.8 ± 2.2 (4)	25.8 ± 5.8 (4)	6.2 ± 0.8 (4)	7.0 ± 0.8 (4)

*Figure in parentheses=number of mice in group.

to favor the view, put forth by Jacobson (*3*), that the active material in bone marrow and spleen is humoral rather than cellular in nature.

Heterologous rat and mouse marrow have been tested in irradiated C57BL mice using the thymic-weight assay. The marrow contained in 6 long bones of 21-day-old normal white rats was suspended in 0.5 ml. of cold Locke's solution and injected intravenously into test mice irradiated in the usual fractionated schedule. The marrow from 4 femurs of 60-day-

TABLE 14.—*Effect of marrow from strain A mice and from rats*

Expt. No.	Material injected	Amount injected	Thymus (*mg.*)	
			Male	Female
1	Strain A marrow.....	4 femurs*.....	34.3 ± 4.1 (5)†	25.1 ± 1.2 (4)
	Rat marrow‡.....	6 bones*.....	21.3 ± 4.2 (5)	32.8 ± 4.0 (5)
	C57BL marrow.....	2 femurs*.....	63.3 ± 3.5 (5)	77.7 ± 4.3 (5)
	Locke's solution.....	0.5 ml.....	22.0 ± 2.0 (5)	49.2 ± 8.3 (4)
2	Strain A marrow.....	.....	24.2 ± 3.0 (6)	36.8 ± 6.1 (6)
	C57BL marrow.....	.....	68.2 ± 4.7 (6)	78.4 ± 3.0 (5)
	Locke's solution.....	.....	29.3 ± 4.1 (6)	29.9 ± 4.0 (6)

*Suspended and injected in 0.5 ml. of Locke's solution.
†Figure in parentheses = number of mice in group.

on lymphoid-tissue recovery in irradiated strain

Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female
90. 4 ± 3. 3 (5)	92. 2 ± 5. 9 (5)	24. 0 ± 0. 1 (5)	19. 2 ± 0. 8 (5)
89. 2 ± 8. 7 (4)	77. 5 ± 5. 6 (4)	21. 5 ± 0. 8 (4)	18. 3 ± 0. 7 (4)
74. 7 ± 2. 7 (10)	86. 7 ± 4. 5 (8)	20. 5 ± 0. 4 (10)	19. 1 ± 0. 6 (8)
84. 5 ± 8. 1 (4)	70. 5 ± 2. 2 (3)	20. 2 ± 1. 3 (4)	17. 9 ± 1. 1 (3)
86. 4 ± 3. 3 (9)	97. 2 ± 8. 5 (8)	23. 2 ± 0. 9 (9)	17. 9 ± 0. 8 (8)
63. 7 ± 3. 7 (4)	80. 4 ± 4. 2 (5)	20. 2 ± 1. 6 (4)	18. 4 ± 0. 6 (5)
81. 0 ± 13.4 (4)	85. 7 ± 10.2 (4)	19. 2 ± 1. 1 (4)	19. 2 ± 0. 8 (4)

old Strong strain A mice was similarly suspended and injected into another group of C57BL mice. Two control groups received homologous C57BL marrow and Locke's solution, respectively. This experiment was repeated, omitting the rat marrow. The results (table 14) are interpreted as essentially negative with respect to both heterologous tissues under these experimental conditions.

on lymphoid-tissue recovery in irradiated strain C57BL mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female	Male	Female
8. 3 ± 1. 1 (5)	9. 2 ± 1. 6 (4)	63. 3 ± 1. 8 (5)	65. 9 ± 3. 9 (4)	21. 7 ± 0. 4 (5)	18. 3 ± 0. 6 (4)
7. 1 ± 1. 5 (5)	10. 2 ± 1. 9 (5)	65. 3 ± 3. 6 (5)	76. 3 ± 0. 6 (5)	21. 8 ± 0. 6 (5)	19. 1 ± 0. 5 (5)
14. 7 ± 1. 9 (5)	12. 4 ± 2. 4 (5)	85. 7 ± 2. 9 (5)	89. 7 ± 2. 1 (5)	22. 5 ± 0. 5 (5)	18. 8 ± 0. 5 (5)
5. 0 ± 0. 6 (5)	10. 2 ± 2. 4 (4)	61. 6 ± 3. 3 (5)	70. 6 ± 5. 6 (4)	21. 2 ± 0. 6 (5)	18. 2 ± 0. 8 (4)
10. 0 ± 1. 3 (6)	12. 1 ± 0. 8 (6)	75. 0 ± 1. 6 (6)	80. 7 ± 5. 0 (6)	22. 5 ± 0. 2 (6)	17. 9 ± 0. 2 (6)
13. 7 ± 2. 2 (6)	12. 1 ± 0. 8 (5)	83. 7 ± 6. 6 (6)	87. 0 ± 6. 6 (5)	22. 5 ± 0. 6 (6)	19. 1 ± 1. 0 (5)
7. 9 ± 1. 3 (6)	9. 0 ± 0. 7 (6)	65. 6 ± 3. 4 (6)	70. 1 ± 3. 8 (6)	22. 5 ± 0. 7 (6)	18. 5 ± 0. 5 (6)

‡Rat donors were 21 days old.

D. *Thigh-shielding and Lymphoid-tissue Recovery in BALB Mice*

BALB (Bagg A) mice are one of the few strains of mice as susceptible as the C57BL strain to the induction of lymphoid tumors by irradiation (*22*). In view of the striking inhibition of tumorigenesis by thigh-shielding in the latter strain, it seemed of interest to test the lymphoid-tissue response to thigh-shielding during the irradiation of BALB mice (table 15). Animals of both sexes, aged 33 ± 3 days old, were treated with 4 doses of 168 r each at 8-day intervals, with one thigh of one group shielded by lead as previously described (*9*). A third group received no irradiation. The animals were sacrificed at days 26, 33, and 50, and lymphoid-tissue weights determined as before. Striking regeneration of the thymus was apparent at day 50, similar to that noted earlier in strain C57BL mice. There was a less pronounced response of superficial lymph-node weights. Of interest is the distinctly greater spleen weight in nonirradiated mice of this strain than in C57BL mice (compare table 13). Body weights are also heavier at the same age, especially in the males.

IX. Modification of the Assay Procedure

A number of attempts have been made to find a shorter assay than the 50-day thymic weight criterion we have used thus far. Cole and Ellis (*23*) have reported favorably on the use of total spleen desoxyribonucleic acid (DNA) content 9 days after total-body irradiation of CF-1 mice.

TABLE 15.—*Effect of thigh-shielding on lymphoid-tissue*

Expt. day	Group	Thymus (*mg.*)	
		Male	Female
26	Age controls	53.5 ± 4.6 (6)*	69.6 ± 2.4 (8)
	X ray; whole body	12.4 ± 2.3 (7)	14.5 ± 3.6 (6)
	X ray; shielded	16.3 ± 2.3 (5)	20.7 ± 3.0 (6)
33	Age controls	50.7 ± 1.9 (6)	57.5 ± 2.1 (7)
	X ray; whole body	24.9 ± 2.4 (5)	31.5 ± 4.4 (6)
	X ray; shielded	31.5 ± 5.4 (4)	47.7 ± 6.8 (5)
50	Age controls	44.9 ± 1.5 (6)	50.4 ± 2.4 (8)
	X ray; whole body	11.5 ± 1.7 (7)	23.5 ± 3.2 (8)
	X ray; shielded	75.6 ± 1.3 (7)	90.1 ± 5.5 (8)

*Figure in parentheses = number of mice in group.

We have not obtained consistent results with this method in C57BL mice after single doses of 200 to 350 r. Moreover, thymic weight has not been significantly greater in marrow-injected or thigh-shielded animals than in controls at 5 to 21 days after single doses of the same magnitude. It seems likely, however, that an optimal combination of X-ray dose and time of sacrifice can be found which will materially reduce the time required for each experiment and thus accelerate our progress in defining the nature of the marrow factor.

Summary and Conclusions

Thymic weight 50 days after the start of fractional total-body irradiation has been used as an assay criterion in strain C57BL mice to obtain additional information concerning the nature and mode of action of the material in mouse bone marrow, which has previously been shown to reduce mortality, promote hematopoietic regeneration, and inhibit lymphoid-tumor development in irradiated mice.

An extensive series of experiments of this type is reported in summary form. Their results support the following tentative conclusions:

1) Marrow cells enclosed in intraperitoneal capsules with a porous end-window gave no evidence that they elaborate a humoral material capable of dissociating from its cell of origin, traversing the window, and acting to promote hematopoietic regeneration in the irradiated host.

regeneration in irradiated BALB mice

Nodes (*mg.*)		Spleen (*mg.*)		Body weight (*gm.*)	
Male	Female	Male	Female	Male	Female
16. 5 ± 1. 8 (6)	15. 9 ± 1. 2 (8)	117. 3 ± 5. 5 (6)	118. 7 ± 4. 3 (8)	25. 4 ± 1. 0 (6)	20. 1 ± 0. 4 (8)
3. 7 ± 0. 6 (7)	4. 3 ± 0. 3 (6)	32. 0 ± 1. 8 (7)	35. 3 ± 3. 4 (6)	15. 4 ± 1. 0 (7)	14. 2 ± 1. 2 (6)
5. 5 ± 0. 8 (5)	4. 5 ± 0. 4 (6)	42. 9 ± 2. 4 (5)	36. 9 ± 1. 2 (6)	18. 7 ± 1. 2 (5)	15. 3 ± 0. 5 (6)
16. 8 ± 1. 9 (6)	19. 6 ± 2. 0 (7)	105. 1 ± 3. 6 (6)	119. 2 ± 3. 2 (7)	24. 4 ± 0. 8 (6)	21. 0 ± 0. 4 (7)
5. 9 ± 1. 4 (5)	4. 2 ± 0. 5 (6)	35. 8 ± 2. 2 (5)	48. 1 ± 6. 0 (6)	19. 2 ± 1. 4 (5)	17. 2 ± 0. 8 (6)
4. 1 ± 0. 5 (4)	4. 0 ± 0. 5 (5)	85. 5 ± 8. 2 (4)	86. 6 ± 14. 6 (5)	19. 3 ± 1. 4 (4)	17. 7 ± 0. 7 (5)
17. 0 ± 1. 7 (6)	18. 8 ± 2. 0 (8)	122. 7 ± 6. 8 (6)	118. 8 ± 3. 3 (8)	27. 5 ± 1. 0 (6)	22. 2 ± 0. 6 (8)
6. 1 ± 1. 0 (7)	5. 6 ± 0. 7 (8)	149. 2 ± 9. 4 (7)	100. 6 ± 5. 5 (8)	23. 3 ± 0. 7 (7)	19. 9 ± 0. 3 (8)
8. 4 ± 0. 4 (7)	10. 7 ± 1. 1 (8)	115. 5 ± 7. 1 (7)	108. 2 ± 5. 4 (8)	24. 4 ± 0. 6 (7)	19. 4 ± 0. 4 (8)

2) Differentially centrifuged mouse bone marrow and spleen cells exhibited activity in the nuclei fraction, which was contaminated by nondisrupted cells to a variable extent, ranging from 10 percent for marrow and 60-day-old spleens to 0.2 percent for 7- to 10-day-old spleens. Activity was inversely related to age of the source material, and not related to the degree of contamination by whole cells. These findings, which confirm those of Cole, Fishler, and Bond (*8*), are not considered entirely conclusive with regard to localization of the active material in the cell nucleus. The cytoplasmic and supernatant fractions were entirely inactive.

3) The effect of thigh-shielding and marrow cell injection is not dependent upon the presence of the spleen.

4) Pretreatment of donor mice with phenylhydrazine or turpentine elicited an intense hyperplasia of the erythroid or myeloid cellular elements, respectively, of the marrow and spleen, but failed to modify activity in the thymic-weight assay. It is tentatively suggested that this result indicates that the active factor resides in more primitive cells of the marrow and spleen.

5) With the exception of fetal liver, all adult and fetal tissues other than marrow or young spleen were inactive. Marrow from strain A (Strong) mice and from rats exhibited no activity by this assay.

6) Homologous marrow incubated *in vitro* with P^{32} and injected intravenously was distributed primarily in the reticuloendothelial tissues, with little in the thymus or blood. Most of the injected activity could not be accounted for, however.

7) Freezing or lyophilization of marrow resulted in inactivation.

References

(*1*) Jacobson, L. O., Marks, E. K., Robson, J. J., Gaston, E. O., and Zirkle, R. E.: The effect of spleen protection on mortality following x-irradiation. J. Lab. & Clin. Med. 34: 1538–1543, 1949.

(*2*) Jacobson, L. O., Simmons, E. L., Marks, E. K., Gaston, E. O., Robson, M. J., and Eldredge, J. H.: Further studies on recovery from radiation injury. J. Lab. & Clin. Med. 37: 683–697, 1951.

(*3*) Jacobson, L. O.: Evidence for a humoral factor (or factors) concerned in recovery from radiation injury: *A review.* Cancer Res. 12: 315–325, 1952.

(*4*) Lorenz, E., Uphoff, D., Reid, T. R., and Shelton, E.: Modification of irradiation injury in mice and guinea pigs by bone marrow injections. J. Nat. Cancer Inst. 12: 197–201, 1951.

(*5*) Congdon, C. C., Uphoff, D., and Lorenz, E.: Modification of acute irradiation injury in mice and guinea pigs by injection of bone marrow: A histopathologic study. J. Nat. Cancer Inst. 13: 73–107, 1952.

(*6*) Lorenz, E., Congdon, C. C., and Uphoff, D.: Prevention of irradiation-induced lymphoid tumors in C57BL mice by spleen protection. J. Nat. Cancer Inst. 14: 291–301, 1953.

(*7*) Lorenz, E., and Congdon, C. C.: Modification of lethal irradiation injury in mice by injection of homologous or heterologous bone. J. Nat. Cancer Inst. 14: 955–965, 1954.

(*8*) Cole, L. J., Fishler, M. C., and Bond, V. P.: Subcellular fractionation of mouse spleen radiation protection activity. Proc. Nat. Acad. Sci. 39: 759–772, 1953.

(*9*) KAPLAN, H. S., and BROWN, M. B.: Further observations on inhibition of lymphoid tumor development by shielding and partial-body irradiation of mice. J. Nat. Cancer Inst. 12: 427–436, 1951.

(*10*) BROWN, M. B., KAPLAN, H. S., WEYMOUTH, P. P., and PAULL, J.: Effect of intravenously injected bone marrow cell suspensions on thymic regeneration in irradiated C57 black mice. Science 117: 693–695, 1953.

(*11*) KAPLAN, H. S., BROWN, M. B., and PAULL, J.: Influence of bone-marrow injections on involution and neoplasia of mouse thymus after systemic irradiation. J. Nat. Cancer Inst. 14: 303–316, 1953.

(*12*) HILFINGER, M. F., Jr., and FERGUSON, J. H.: Experimental transfusions of bone marrow emulsion into rabbits after total body irradiation. (Abstract.) Am. J. Path. 27: 675, 1951.

(*13*) STORER, J. B., LUSHBAUGH, C. C., and FURCHNER, J. E.: The protective effect of shielded ectopic bone marrow against total body x-radiation. J. Lab. & Clin. Med. 40: 355–366, 1952.

(*14*) HILFINGER, M. F., Jr., FERGUSON, J. H., and RIEMENSCHNEIDER, P. A.: The effect of homologous bone marrow emulsion on rabbits after total body irradiation. J. Lab. & Clin. Med. 42: 581–591, 1953.

(*15*) SCHNEIDER, W. C., and HOGEBOOM, G. H.: Cytochemical studies of mammalian tissues: the isolation of cell components by differential centrifugation: *A review.* Cancer Res. 11: 1–22, 1951.

(*16*) AVERY, O. T., MACLEOD, C. M. and MCCARTY, M.: Studies on the chemical nature of the substance inducing transformation of pneumococcal types. Induction of transformation by a desoxyribonucleic acid fraction isolated from pneumococcus type III. J. Exper. Med. 79: 137–158, 1944.

(*17*) ZAMENHOF, S., ALEXANDER, H. E., and LEIDY, G.: Studies on the chemistry of the transforming activity. I. Resistance to chemical and physical agents. J. Exper. Med. 98: 373–397, 1953.

(*18*) DIXON, W. J., and MASSEY, F. J., Jr.: Introduction to Statistical Analysis. New York, McGraw-Hill Book Company, Inc., 1951, p. 239.

(*19*) KAPLAN, H. S., and PAULL, J.: Genetic modification of response to spleen shielding in irradiated mice. Proc. Soc. Exper. Biol. & Med. 79: 670–672, 1952.

(*20*) KAPLAN, H. S., and BROWN, M. B.: Testosterone prevention of post-irradiation lymphomas in C57 black mice. Cancer Res. 12: 445–447, 1952.

(*21*) GARDNER, W. U., DOUGHERTY, T. F., and WILLIAMS, W. L.: Lymphoid tumors in mice receiving steroid hormones. Cancer Res. 4: 73–87, 1944.

(*22*) KIRSCHBAUM, A., SHAPIRO, J. R., and MIXER, H. W.: Synergistic action of leukemogenic agents. Cancer Res. 13: 262–268, 1953.

(*23*) COLE, L. J., and ELLIS, M. E.: Spleen desoxyribonucleic acid content as an index of recovery in x-irradiated mice treated with spleen homogenates. Cancer Res. 14: 738–744, 1954.

The Time Factor in Inhibition of Lymphoid-Tumor Development by Injection of Marrow Cell Suspensions into Irradiated C57BL Mice[1, 2, 3]

HENRY S. KAPLAN, LINCOLN E. MOSES, MARY B. BROWN, C. SUSAN NAGAREDA, *and* BARBARA B. HIRSCH, *Departments of Radiology and Public Health, Stanford University School of Medicine, San Francisco, Calif.*

Earlier studies have demonstrated that lymphoid-tumor development, which occurs in high incidence in systemically irradiated strain C57BL mice (*1*), may be very effectively inhibited by shielding the thigh (*2*, *3*) or the exteriorized spleen (*4*) during irradiation, or by the intravenous injection of homologous bone-marrow cells after irradiation of unshielded animals (*5*). Since the animals are known to suffer the same degree of initial injury as irradiated controls, the effect appeared to involve a reversal of the tumorigenic process in its early phase. There has been little information published concerning either the existence or duration of a phase of reversibility in tumor development. This paper presents the results of an experiment in which graded intervals of time were permitted to elapse between the completion of irradiation and the injection of marrow.

Materials and Methods

Randomized groups of strain C57BL mice of both sexes were given 4 total-body doses of 168 r each at 8-day intervals, starting at 33 ± 3 days of age. A control group then received an 0.5 ml. intravenous injection of Locke's solution. Marrow cell suspensions were prepared as before (*5*) from the femoral marrow of 60-day-old donor mice taken from the same litters as the recipients. Each test animal received the marrow from both femurs of a single donor in 0.5 ml. of Locke's solution as a single intravenous injection at exponentially graded time intervals (1/16 day, ⅛ day, ¼ day—16, 32 days) following the last X-ray treatment. An additional group received the same amount of marrow intraperitoneally at the 1/16-day (1½ hour) time interval.

The animals were maintained under identical conditions, with free access to Purina laboratory chow and water. When found moribund or dead, they were routinely autopsied and pertinent tissues were fixed for histologic study, except where typical lymphomas were apparent grossly.

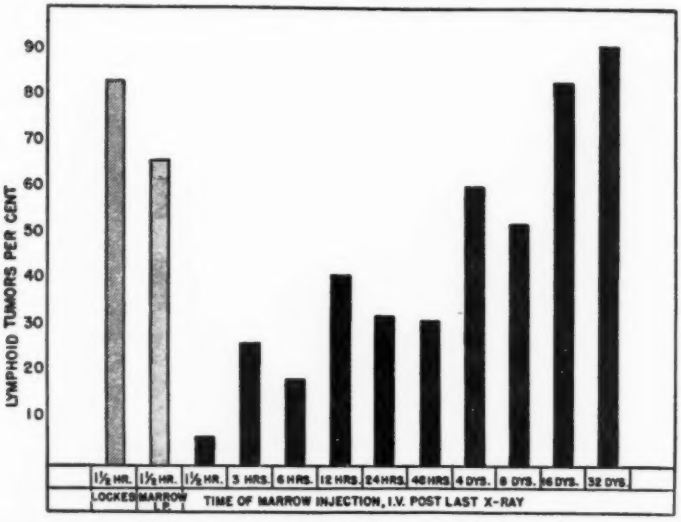
[1] Received for publication October 27, 1954.

[2] Presented in part in a Symposium on Leukemia at the Annual Meeting of the American Association for Cancer Research, Inc., Atlantic City, April 10-12, 1954.

[3] These studies were supported by grants-in-aid from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and the Jane Coffin Childs Memorial Fund for Medical Research.

Results

The results are presented in table 1 and text-figure 1. There was almost no inhibition of tumor formation when marrow was given intraperitoneally. Delayed intravenous injection resulted in a graded response of tumor incidence. The percentage of tumors in each group and sex was transformed by the method of anglits (*6*). From this transformation, the linear regression on log delay was then calculated for each sex. The data plotted in this way are shown in text-figure 2, together with the best fitting lines having common slope. The χ^2 test of homogeneity indicates that the model of two linear regressions having common slope



TEXT-FIGURE 1.—Time factor in lymphoid-tumor inhibition by homologous bone-marrow injections in irradiated strain C57BL mice.

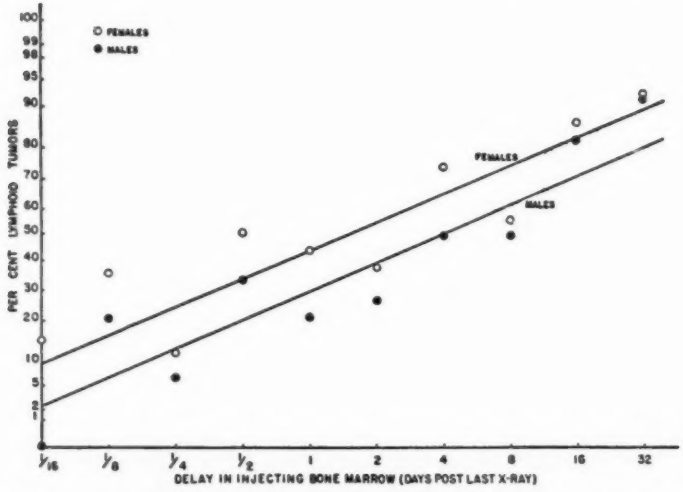
is tenable ($\chi^2_{17}=21.51$, $P>0.10$). Thus the effect of delayed marrow injection may be considered in terms of doubling or halving the delay, rather than on the basis of time units such as days or hours.

The sexes appeared to be essentially equally affected by delay in the receipt of bone marrow under these experimental conditions. It is of interest that a greater tendency of females to develop radiation-induced lymphomas is clearly brought out in this experiment. Earlier studies have revealed no sex difference in final tumor incidence in this strain except when quite large numbers of mice were used, in which case a small but significant indication of greater susceptibility in females was obtained (*1*). In text-figure 2, it may be seen that at each of the 9 delay levels the percentage of females with tumors exceeds that of the males. The difference in susceptibility is measured by the horizontal distance between the two lines, which is 1.37 steps (a step being a doubling of delay) with a standard error of $\pm$ 0.25 steps. The sex difference is therefore statistically sig-

TABLE 1.—*Effect of delayed injections of bone marrow on lymphoid-tumor incidence in irradiated C57BL mice*

Group	Injection	Time of injection (post last X ray)	Net number of mice			Mice with lymphomas						Mice without lymphomas			Mean latent period (*days*)		
						Male		Female		Total							
			Male	Female	Total	No.	Percent	No.	Percent	No.	Percent	Male	Female	Total	Male	Female	Total
I	Locke's	1½ hours	13	18	31	11	85	15	83	26	84	2	3	5	193	158	173
II	Marrow	1½ hours	17	14	31	0	0	2	14	2	6	17	12	29	.....	153	153
III	Marrow	3 hours	19	14	33	4	21	5	36	9	27	15	9	24	188	247	221
IV	Marrow	6 hours	15	17	32	1	7	5	29	6	19	14	12	26	165	183	180
V	Marrow	12 hours	15	16	31	5	33	8	50	13	42	10	8	18	229	214	220
VI	Marrow	24 hours	14	16	30	3	21	7	44	10	33	11	9	20	157	167	164
VII	Marrow	48 hours	15	16	31	4	27	6	37	10	32	11	10	21	179	171	174
VIII	Marrow	4 days	16	15	31	8	50	11	73	19	61	8	4	12	200	164	179
IX	Marrow	8 days	12	18	30	6	50	10	56	16	53	6	8	14	192	167	176
X	Marrow	16 days	17	15	32	14	82	13	87	27	84	3	2	5	184	174	179
XI	Marrow	32 days	12	14	26	11	92	13	93	24	92	1	1	2	205	158	179
XII	Marrow	1½ hours, I. P.	25	5	30	17	68	3	60	20	67	8	2	10	179	175	178

Vol. 15, No. 4, February 1955
320174—55——15



TEXT-FIGURE 2.—Percent of tumor response (as 2 $\sin^{-1}\sqrt{P}$) plotted as a function of log-delay in injecting bone marrow into irradiated strain C57BL mice.

nificant. Roughly, in order to reduce the incidence of tumors in females to that in males at this radiation dose level, it would be necessary to inject the marrow into females earlier by about 1.37 ± 0.5 steps.

Discussion

The time required for the marrow to accomplish its inhibitory effect, measured from the time of its injection, is of course unknown, since the site and precise mechanism of its action are not established. The total time required for reversibility of tumorigenesis is thus actually the sum of the known delay of injection plus this unknown time increment. It seems a fair guess that this time interval for marrow action is of the order of minutes, or at most a few hours, rather than a matter of days or even several hours; if, for example, this time interval were 6 hours then the logarithmic difference between 3 hours and 1½ hours would actually be:

$$\log_2 (6+3) - \log_2 (6+1\tfrac{1}{2}) = .263$$

and the logarithmic difference between 2 days and 1 day would be:

$$\log_2 (48 + 6) - \log_2 (24+6) = .848.$$

But a good fit was obtained assuming these intervals to be *equal* logarithmically, arguing strongly against such a long postinjection time interval for the action of the marrow.

Over the range of time intervals studied (1/16 day to 32 days) there was a gradual decrease in the inhibitory effect of injected bone marrow as the delay between last irradiation and injection was allowed to increase. After 16 to 32 days the inhibitory effect was essentially lost.

These results are most readily interpreted in terms of a gradual decrease in the probability that the marrow will successfully inhibit tumorigenesis. It is clear that however the injected marrow may interrupt and reverse the course of tumorigenesis, the *time* of this reversal must be early, a matter of, at most, days after the last irradiation. The probability that in a given animal the tumorigenic process is still reversible by marrow injection at any given time can be estimated from text-figure 2.

Summary

Groups of strain C57BL mice were given periodic total-body irradiation and injected intravenously with homologous bone-marrow cell suspensions at graded intervals of time thereafter. Lymphoid-tumor incidence was inhibited maximally under these conditions when injection was carried out within 1½ hours after the last X-ray dose. Inhibition decreased in linear relation to log delay time and was essentially completely lost after a delay of about 16 days. It is inferred that the time when the potentiality of lymphoid-tumor development becomes irreversible in a population of irradiated animals is similarly statistically distributed, and it is suggested that the time required for the injected marrow to exert its inhibitory action, though unknown, is more likely of the order of minutes or perhaps a few hours rather than a matter of days or many hours.

Of auxiliary interest is the distinct sex difference in susceptibility revealed under the conditions of this experiment. Females developed more tumors than males at all delay intervals studied, or viewed differently, they would require earlier marrow injection than males for equal tumor incidence, the difference being about 1.37 steps where a step denotes a doubling of the delay.

References

(*1*) KAPLAN, H. S., and BROWN, M. B.: A quantitative dose-response study of lymphoid-tumor development in irradiated C57 black mice. J. Nat. Cancer Inst. 13: 185–208, 1952.

(*2*) ———: Protection against radiation-induced lymphoma development by shielding and partial-body irradiation of mice. Cancer Res. 12: 441–444, 1952.

(*3*) ———: Further observations on inhibition of lymphoid tumor development by shielding and partial-body irradiation of mice. J. Nat. Cancer Inst. 12: 427–436, 1951.

(*4*) LORENZ, E., CONGDON, C. C., and UPHOFF, D.: Prevention of irradiation-induced lymphoid tumors in C57BL mice by spleen protection. J. Nat. Cancer Inst. 14: 291–301, 1953.

(*5*) KAPLAN, H. S., BROWN, M. B., and PAULL, J.: Influence of bone-marrow injections on involution and neoplasia of mouse thymus after systemic irradiation. J. Nat. Cancer Inst. 14: 303–315, 1953.

(*6*) HALD, A.: Statistical Tables and Formulas. New York, John Wiley & Son, 1952, table 12.

Nucleic Acid Content of the Thymic Cells of Normal and Irradiated C57BL Mice[1,2]

PATRICIA P. WEYMOUTH,[3] NORMAN E. DELFEL, RUTH J. DOELL, HELENE L. STEINBOCK, *and* HENRY S. KAPLAN, *Department of Radiology, Stanford University School of Medicine, San Francisco, Calif.*

The biological system utilized in this investigation has previously been characterized as follows (*1–3*): Strain C57BL mice, which develop spontaneous lymphoid tumors very infrequently, may be induced to yield such tumors in high incidence 4 to 10 months after suitable doses of total-body X irradiation. The incidence of tumors is greatly decreased by shielding one thigh during irradiation; this inhibitory affect of shielding can also be produced by the intravenous injection of homologous bone-marrow cell suspensions into unshielded animals after irradiation. The tumors are all lymphosarcomas or lymphatic leukemias which almost invariably originate in the thymus gland in this strain. In studies of the biochemical events of possible importance in the tumor-induction process, advantage may be taken of these phenomena in two ways: *a*) attention may properly be focused on a single tissue, the thymus; and *b*) an internal control may be used to test the relevance of any biochemical aberrations encountered, by comparing the results in thigh-shielded (or marrow-injected) animals with those in unshielded irradiated animals.

For many reasons, it seemed desirable to start with an investigation of the effect of irradiation on thymic nucleic acids. In our initial studies, a modification of the Schmidt-Thannhauser method (*4*) was used to obtain data on nucleic acid *concentration*, expressed as DNAP[4] and RNAP per mg. wet weight of thymus. Irradiation was followed by a sharp and sustained decrease in concentration of DNAP, with little or no change in RNAP concentration (*5*). It became apparent, however, that the results could not be interpreted in this form, because the concentrations of DNA and RNA might vary independently as a result of changes in tissue cellularity, hydration, and relative nuclear and cytoplasmic volumes. It was therefore decided to repeat this work on the basis of nucleic acid

[1] Received for publication October 27, 1954.

[2] This investigation was supported by grants-in-aid from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service, and the Jane Coffin Childs Memorial Fund for Medical Reseach.

[3] *Present address:* Clarkson College, Potsdam, N. Y.

[4] The following abbreviations are used: DNAP=desoxyribonucleic acid phosphorus; RNAP=ribonucleic acid phosphorus; DNA=desoxyribonucleic acid; RNA=ribonucleic acid. The RNAP fraction also contains small amounts of phosphorus from phosphoproteins and possibly other sources.

content per cell. Two approaches have been employed: *a*) histochemical estimations of the DNA content of individual thymic cell nuclei stained by the Feulgen method have been made by Dr. William Carnes and his co-workers, using the microphotometric technique (*6*); and *b*) chemical determinations of DNA and RNA in aliquots of counted thymic cell suspensions have been made, which permit calculation of average DNA and RNA content per cell. The present report is concerned with two such experiments in which different methods for measuring DNA and RNA have given similar results.

Materials and Methods

Strain C57BL mice of both sexes were randomized into three series: 1) an unirradiated control group; 2) an irradiated unshielded group; and 3) an irradiated thigh-shielded group. Treatment was begun when the mice were 33 ± 3 days of age. Irradiation was given in 4 doses of 168 r each at intervals of 8 days, the last treatment therefore being given on day 25. Physical factors and technique of irradiation were as previously described (*3*).

In Experiment 1, groups of 6 to 14 animals from each of the three series were sacrificed at days 29, 42, 53, 65, 85, 99, and 120. In Experiment 2, equal numbers of male and female mice (usually 3 of each) from each series were sacrificed on days 26 (30 hours after the last irradiation), 30, 45, and 106.

The thymus of each mouse was rapidly dissected free of fat and connective tissue, weighed on a torsion balance, frozen with dry ice, and stored until use at dry-ice temperatures.[5] In the irradiated groups of Experiment 2 at days 26 and 30, it was necessary to pool two thymus glands from mice of the same sex for each determination due to their low weight; all other determinations were done on single tissues. After thawing at 4° C., each thymus was pressed through an 80-mesh brass screen into iced Locke's solution. The resulting cell suspensions were relatively free of clumping and matrix on microscopic examination. The number of cells per unit volume was determined in a hemacytometer, with 0.02-percent gentian violet in 3-percent acetic acid as the diluent. Over 500 cells were counted in each of two aliquots of each suspension. A third aliquot, containing the equivalent of 12 to 25 mg. of original tissue, was diluted with one volume of cold 95-percent alcohol and homogenized in a glass microhomogenizer at 4° C. Lipids and acid-soluble components were removed by extraction once with cold 95-percent alcohol, twice with ether:alcohol (1:3) at 60° C., and three times with cold 5-percent trichloroacetic acid.

In Experiment 1, nucleic acids were determined by a slight modification of the Schmidt-Thannhauser procedure, as previously described (*5*).

[5] Although pancreatic ribonuclease apparently remains active during tissue storage at subzero temperatures (Professor F. W. Allen—personal communication) there is no evidence that the corresponding enzyme in tissues other than pancreas behaves similarly. In any case, this possible source of error could have affected only the absolute values of RNA per cell, and not the relative amounts found in the thymus glands of the three groups, all of which were stored for the same duration.

However, the work of Drasher (*7*) and others emphasized the need for checking the accuracy of this method. Prior to Experiment 2, therefore, parallel aliquots of thymic cell suspensions were analyzed for DNA and RNA by the methods of Schmidt and Thannhauser, Ogur and Rosen (*8*), and Schneider (*9*). It was found that the "RNA" fractions obtained by the first two methods were contaminated to the extent of about 15 percent by material which gave the diphenylamine color reaction and was therefore presumably DNA. Accordingly, in Experiment 2 the nucleic acids were extracted by the method of Schneider (*9*). Aliquots of these extracts, containing the equivalent of 12 mg. wet weight of thymus per 0.1 cc., were analyzed for RNA by the method of Hahn and von Euler (*10*) using a ribose standard (Nutritional Biochemicals Company), and for DNA by the method of Dische (*11*) using commercial thymonucleic acid (Nutritional Biochemicals Company) as a standard, with a 10-minute heating period. For the measurement of optical density, the solutions were read in a Beckman model DU spectrophotometer using microcuvettes.

As an appendix to Experiment 1, similar determinations were made on the C43 transplantable lymphosarcoma 15 days after subcutaneous implantation into 55-day-old C57BL mice. The uninvolved thymus of each tumor-bearing animal was used as a control tissue.

Results

The results of the two experiments are presented in tables 1 and 2, respectively. It may be seen that they are qualitatively quite similar. In view of the technical considerations mentioned above, however, the results of Experiment 2 are considered to be more accurate.

The amount of DNA per thymic cell in the nonirradiated control animals was essentially constant throughout the course of both experiments with the exception of the values for day 106, Experiment 2. It is felt that the higher values observed here were due to some bias in the cell preparation and counting procedure, since the amount of RNA per cell in the control group also appears to be somewhat elevated. There was a slight, but statistically nonsignificant elevation of DNA content in both irradiated groups.

Over the age range studied, RNA content of the thymus cells of the control mice was essentially constant. Irradiation caused a striking change in average RNA content (text-fig. 1). Both the shielded and unshielded irradiated groups exhibited a twofold to threefold increase within the first few days after treatment. This abnormally high level of RNA content was sustained throughout the experiment in the unshielded, irradiated animals. Induced tumors encountered in some of the unshielded mice near the end of each experiment also had significantly high values of RNA per cell. In the shielded animals, however, RNA per cell returned to normal by day 45.

Earlier studies have revealed that thymic weight also returns to normal in the shielded animals at about the same time (text-fig. 2), through the

TABLE 1.—*Average DNAP and RNAP per cell in thymus of strain C57BL mice*

Day	Expt.	DNAP						RNAP					
		Control		Irradiated		Thigh-shielded		Control		Irradiated		Thigh-shielded	
		No.	$\overline{X}$*	No.	$\overline{X}$*	No.	$\overline{X}$*	No.	$\overline{X}$†	No.	$\overline{X}$†	No.	$\overline{X}$†
29	117a	8	6. 46	6	6. 78	7	7. 38	8	1. 63	5	5. 55	8	5. 62
	117b	4	4. 97	8	5. 26	6	5. 43	4	1. 29	8	5. 48	6	4. 61
	135a	4	7. 04	4	6. 56	4	6. 27	4	1. 38	4	5. 45	4	5. 98
	135b	4	6. 22	4	6. 94	3	6. 46	4	1. 68	4	4. 65	3	4. 11
42	117	6	6. 79	6	6. 12	6	6. 85	6	1. 62	6	2. 11	6	1. 81
	135a	4	5. 86	4	6. 31	2	6. 94	4	1. 69	4	3. 18	2	2. 20
	135b	4	6. 23	3	7. 03	2	6. 57	4	1. 87	3	3. 54	2	1. 90
55	128	.....	.....	12	5. 37	6	6. 19	.....	.....	12	2. 23	6	1. 43
84	117	4	5. 98	7	6. 22	.....	.....	3	1. 40	6	3. 32	.....	.....
	128	10	7. 01	11	8. 70	6	8. 66	10	2. 31	10	4. 63	6	2. 79
97	117	6	5. 26	13	6. 32	.....	.....	7	1. 44	14	3. 18	.....	.....
	128	.....	.....	10	6. 90	6	5. 82	.....	.....	11	3. 15	6	1. 48
120	128	8	5. 60	11	6. 24	6	5. 95	7	1. 59	12	4. 03	6	1. 68

*Mean μg. per cell $\times 10^{-7}$.
†Mean μg. per cell $\times 10^{-7}$.

TABLE 2.—*Average RNA per cell, DNA per cell and RNA/DNA values in thymus of strain C57BL mice (Experiment 2)*

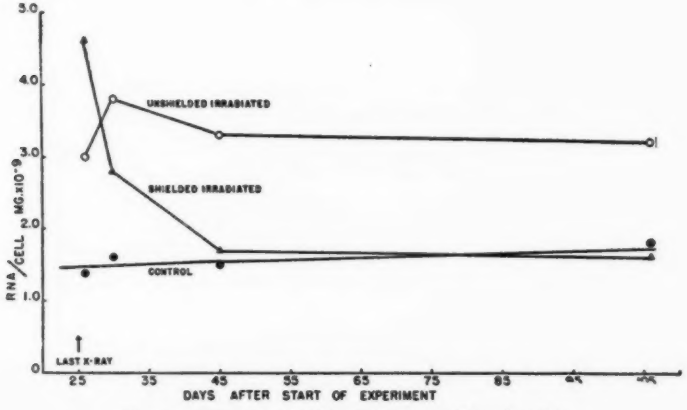
Day	Group	No. of determinations	RNA/cell, ($mg. \times 10^{-9}$)	DNA/cell ($mg. \times 10^{-9}$)	RNA/DNA	Thymic weight ($mg.$)
26	Control	5	1.4 ± 0.2*	7.6 ± 0.4	0.18 ± 0.02	64. 4
	Unshielded	5	3.0 ± 0.3	8.7 ± 0.7	0.34 ± 0.02	17. 3
	Shielded	7	4.8 ± 0.5	9.7 ± 0.5	0.49 ± 0.04	15. 6
30	Control	6	1.6 ± 0.1	7.6 ± 0.2	0.21 ± 0.01	50. 8
	Unshielded	6	3.8 ± 0.2	9.4 ± 0.6	0.43 ± 0.03	23. 8
	Shielded	6	2.8 ± 0.4	7.4 ± 0.2	0.38 ± 0.06	27. 4
45	Control	6	1.5 ± 0.1	7.1 ± 0.2	0.21 ± 0.01	53. 2
	Unshielded	6	3.3 ± 0.4	8.3 ± 0.3	0.40 ± 0.06	25. 2
	Shielded	6	1.7 ± 0.2	7.2 ± 0.3	0.24 ± 0.02	53. 4
106	Control	8	1.8 ± 0.1	9.3 ± 0.4	0.20 ± 0.02	38. 8
	Unshielded	8	3.2 ± 0.8†	8.9 ± 0.4	0.35 ± 0.05	54. 0
	Shielded	8	1.6 ± 0.2	8.6 ± 0.5	0.18 ± 0.02	38. 7

*Figure following ± sign is standard error of mean, given by $\sqrt{\frac{\Sigma(\bar{x}-x)^2}{N(n-1)}}$

†This group contained two thymic lymphosarcomas.

influence of a radiosensitive material in bone marrow, some of which is protected in the femoral marrow of the thigh-shielded animals (*3*). In direct contrast, the unshielded irradiated mice exhibit a very slow recovery of thymic weight. The high mean thymic weight in this group at day 106 of Experiment 2 is due to the presence of early tumors in some mice of the group, and not to normal thymic regeneration.

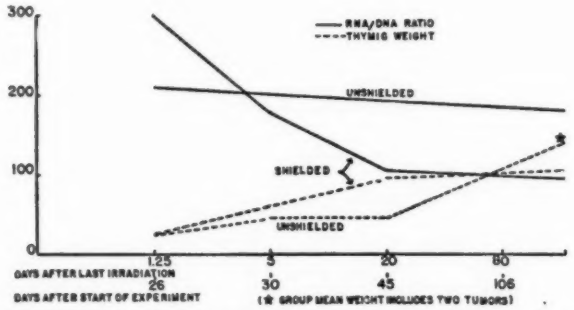
Since DNA per cell was essentially constant, the ratio of RNA to DNA follows the pattern of RNA per cell. As Davidson and Leslie (*12*) pointed out, when DNA per cell is constant, RNA per cell may be conveniently expressed in terms of this ratio, derived from tissue-concentration data, obviating the need for cell counts. Indeed, the ratios calculated from our



TEXT-FIGURE 1.—RNA content per thymic cell

earlier data on thymic DNA and RNA concentration (*5*) are in good agreement with those obtained in Experiment 2.

Preliminary data indicate that the intravenous injection of homologous bone-marrow cell suspensions after total-body irradiation results in a restoration of RNA content of the thymic cells to normal by about day 45 to day 50, in essentially the same way as observed after thigh-shielding. Cell suspensions of the C43 transplantable lymphosarcoma exhibited levels of DNA per cell within the same range as those of the control thymus cells of the hosts, whereas RNA per cell in the tumor cells was greatly elevated, averaging about 900 percent of normal.



TEXT-FIGURE 2.—Semilogarithmic plot of the RNA/DNA ratio and thymic weight of shielded and unshielded, irradiated mice expressed as percent of the control value.

Discussion

The shielded animals suffer the same initial radiation damage, but recover rapidly and do not develop the high incidence of tumors noted in the unshielded, irradiated animals. It seems reasonable to assume that comparison of these two groups might permit differentiation between the biochemical responses which result from nonspecific radiation effects and those which are related to the tumorigenic action of the X rays. The fact that the irradiated thymus cells of the unshielded mice exhibit a persistent elevation of RNA content may be intimately related to the lymphoid-tumor-induction process.

Parallel histologic studies [6] of the thymus glands of shielded and unshielded mice subjected to the same dose and technique of irradiation appear to correlate with the biochemical findings reported in this paper. The most prevalent cell in the normal thymus is the thymocyte, which is essentially indistinguishable from the small, mature lymphocyte. The thymic cortex is very densely packed with thymocytes, and they are also quite numerous in the medulla. Although they have a rather small nucleus, thymocytes have so little cytoplasm that the nucleus occupies most of the volume of the cell. In view of their overwhelming predominance in the

[6] H. S. Kaplan and M. B. Brown—unpublished observations.

normal thymus, it seems reasonable to attribute the low RNA/DNA ratio in the normal gland to a low RNA content in these cells, which is consistent with their minimal amount of cytoplasm.

It has long been known that the thymocyte is exceedingly radiosensitive. Pyknosis and disintegration of these cells is prominent within 24 hours after irradiation. At 3 to 5 days, depending on X-ray dose, they are almost completely wiped out in the cortex, and very sparse in the medulla. Beginning at about 6 to 8 days after treatment, thymocytes rapidly repopulate both the cortex and medulla, and the over-all cellularity of the thymus soon returns to essentially normal levels. During the initial phase of injury, while the thymus is so markedly depleted of its dominant cell-type, the radioresistant cells which persist include epithelial and connective-tissue cells and macrophages, all of which have much greater amounts of cytoplasm than the thymocytes and may be presumed to contain more RNA. The increased RNA content of thymic cell suspensions within the first few days after irradiation may be regarded, therefore, as a biochemical expression of this striking, though transient, change in cell population in the irradiated thymus.

The thymocytes which first reappear during the phase of regeneration resemble large rather than small lymphocytes, with a correspondingly greater diameter and distinctly more cytoplasm. In the shielded animals, this cell type is gradually replaced over a period of several days by the typical thymocyte or small lymphocyte and concurrently the mass of the gland increases to normal levels. In the unshielded, irradiated mice, however, the large thymocyte tends to remain the dominant cell-type for an indefinitely long interval. It is probable that these larger cells, with their more abundant cytoplasm, also contain more RNA than the small lymphocytes. If this is the case, their presence in large numbers would explain the sustained elevation of RNA content in the thymic cell suspensions from the unshielded irradiated group. Such an interpretation of the atypical RNA values, which links them to an altered thymic cell population, does not, of course, diminish the possible significance in tumorigenesis of either the biochemical or the histologic observations.

On the basis of essentially indirect evidence, Caspersson (*13*) has suggested that RNA plays a role in protein synthesis. Direct experimental support for this view has come from the recent work of Gale and Folkes (*14*). Although their studies on disrupted staphylococcal cells *in vitro* establish protein synthesis as a function of RNA and DNA in bacteria, this conclusion cannot yet be extrapolated to mammalian-tissue cells. Nonetheless, they indicate the need for an investigation of protein synthesis in thymic cells of shielded and unshielded irradiated mice at the same time intervals as were used in the present experiments. In addition, it would seem necessary to determine whether a sustained elevation of RNA per cell occurs in the thymus only under circumstances which lead to tumor formation, and to exclude the possibility that a similar response may follow treatment with nontumorigenic thymolytic agents such as the adrenal oxy-steroids or testosterone.

It is is of some interest to compare our data with other published observations. In Experiment 2, the mean value of DNA per cell for normal thymus (omitting day 106) ranged from 7.1×10^{-9} to 7.6×10^{-9} mg., which is slightly higher than the value of 6.5×10^{-9} mg. per splenic-cell nucleus noted by Arnesen, Goldsmith, and Dulaney (*15*) and by Petermann and Schneider (*16*), using a procedure which involved isolation of cell nuclei. Davidson and Leslie (*12*), in a review of the literature to 1950, cite the figure 6.4×10^{-9} mg. per nucleus as representative of normal thymus cells, presumably from calf thymus.

Following irradiation, the cellular content of DNA fluctuated somewhat but did not deviate significantly from control levels. This confirms the earlier observations of Harrington and Lavik (*17*) that DNA per rat-thymus cell was essentially unchanged following an LD_{50} dose of radioactive phosphorus. An accumulation of pentose nucleotides in the cytoplasm of irradiated normal and tumor cells was observed by Mitchell (*18*) several years ago, using ultraviolet microscopy. Recently, Gardella and Lichtler (*19*) reported that DNA content of ascites tumor cells remained unchanged over a 48-hour period following an X-ray dose of 1,000 r; RNA content was unaffected during the first 6 hours but had almost doubled by 17 hours after irradiation, a finding with which our data are in very good agreement.

Finally, the question arises whether these patterns of nucleic acid content also occur in other lymphomas and leukemias, and perhaps in other types of tumors. Carnes, Forker, and Lier (*20*) observed no significant difference between the DNA content of individual cells of early radiation-induced thymic lymphosarcomas and that of normal or irradiated thymus cells. Transplantable lymphosarcomas may or may not exhibit abnormally high levels of DNA. In the cells of the L #1 tumor, Carnes, Weissman, and Goldberg (*21*) observed by microphotometric analysis a high frequency of departure from normal diploid DNA content. This result has recently been confirmed for tumor L #1 by Shelton (*22*) and by Petrakis and Folstad (*23*); these investigators also studied another transplantable lymphosarcoma, L #2, the cells of which exhibited essentially normal DNA content. It would appear therefore that abnormal levels of ploidy with increase in nuclear content of DNA are not an essential concomitant of the transformation of cells to malignant lymphocytes.

The ratios of RNAP/DNAP calculated for tumors L #1 and L #2 from the data of Shelton (*22*) are 0.38 and 0.67, respectively, both of which are high in comparison with our values in normal thymus cells. Other investigators, using cell-fractionation techniques, have noted elevated levels of RNA per nucleus in leukemic spleen cells (*16*) and in an ascites lymphoma (*24*). Thus, the available evidence suggests that an increase in cellular RNA content may be a general characteristic of lymphosarcomas and lymphatic leukemias. There is little evidence on this point with respect to other types of neoplasms. During azo-dye carcinogenesis, however, low rather than high ratios of RNA/DNA in rat-liver cells have been reported (*25*).

Summary

Young strain C57BL mice were divided into three groups: a nonirradiated control group; a thigh-shielded, irradiated group; and an unshielded, irradiated group. They were sacrificed at serial intervals for the determination of RNA and DNA per cell in thymic cell suspensions.

The level of DNA per cell did not vary significantly with age or treatment. RNA values per cell remained essentially constant with time in the control group. There was roughly a threefold increase in RNA per cell 1 to 5 days after irradiation, with a rather prompt return to normal in the shielded group. In contrast, the levels of RNA per cell, and the ratio RNA/DNA, remained elevated over the entire course of the experiment in the unshielded irradiated group. Inasmuch as the shielded animals are known to be refractory to thymic lymphoid-tumor development, whereas the unshielded irradiated animals are highly suspectible, it is suggested that the sustained elevation of thymic cell RNA content may be related in some fundamental way to the genesis of experimental lymphoid tumors.

In parallel studies on the C43 transplantable lymphosarcoma, in which the normal thymus cells of the host C57BL mice were used as a control tissue, levels of RNA per cell as high as 900 percent of normal were noted.

References

(*1*) KAPLAN, H. S.: Comparative susceptibility of the lymphoid tissues of strain C57 black mice to the induction of lymphoid tumors by irradiation. J. Nat. Cancer Inst. 8: 191–197, 1948.

(*2*) KAPLAN, H. S., and BROWN, M. B.: Further observations on inhibition of lymphoid tumor development by shielding and partial-body irradiation of mice. J. Nat. Cancer Inst. 12: 427–436, 1951.

(*3*) KAPLAN, H. S., BROWN, M. B., and PAULL, J.: Influence of bone-marrow injections on involution and neoplasia of mouse thymus after systemic irradiation. J. Nat. Cancer Inst. 14: 303–316, 1953.

(*4*) SCHMIDT, G., and THANNHAUSER, S. J.: Method for determination of desoxyribonucleic acid, ribonucleic acid and phosphoproteins in animal tissues. J. Biol. Chem. 161: 83–89, 1945.

(*5*) WEYMOUTH, P. P., and KAPLAN, H. S.: Effect of irradiation on lymphoid tissue nucleic acid in C57BL mice. Cancer Res. 12: 680–683, 1952.

(*6*) CARNES, W. H., FORKER, B. R., and LIER, E.: Relation of nuclear desoxypentose nucleic acid content of mouse thymus cells to recovery from X-irradiation. (Abstract.) Fed. Proc. 13: 425, 1954.

(*7*) DRASHER, M. L.: A criticism of the indiscriminate use of the Schmidt-Thannhauser method for the fractionation of nucleic acids in biological material. Science 118: 181–183, 1953.

(*8*) OGUR, M., and ROSEN, G.: The nucleic acids of plant tissues. I. The extraction and estimation of desoxypentose nucleic acid and pentose nucleic acid. Arch. Biochem. 25: 262–276, 1950.

(*9*) SCHNEIDER, W. C.: Phosphorus compounds in animal tissues. I. Extraction and estimation of desoxypentose nucleic acid and of pentose nucleic acid. J. Biol. Chem. 161: 293–303, 1945.

(*10*) HAHN, L., and VON EULER, H.: Eine neue Methode zur Bestimmung von Ribonukleinsäure in anwesenheit von Desoxyribonukleinsäure. Ark. kem., min., geol. 22A, #23 (11 pp.), 1946.

(*11*) DISCHE, Z.; Uber einige neue Charakteristische Farbreaktionen der Thymonukleinsäure und eine Mikromethode zur Bestimmung derselben in Tierischen Organen mit Hilfe dieser Reaktionen. Mikrochemie 8: 4–32, 1930.

(*12*) DAVIDSON, J. N., and LESLIE, I.: Nucleic acids in relation to tissue growth: a review. Cancer Res. 10: 587–594, 1950.

(*13*) CASPERSSON, T.: The relations between nucleic acid and protein synthesis. Symp. Soc. Exper. Biol. 1: 127–151, 1947.

(*14*) GALE, E. F., and FOLKES, J. P.: Effect of nucleic acids on protein synthesis and amino-acid incorporation in disrupted staphylococcal cells. Nature 173: 1223–1227, 1954.

(*15*) ARNESEN, K., GOLDSMITH, Y., and DULANEY, A. D.: Antigenic properties of nuclei segregated from spleens of normal and leukemic mice. Cancer Res. 9: 669–671, 1949.

(*16*) PETERMANN, M. L., and SCHNEIDER, R. M.: Nuclei from normal and leukemic mouse spleen. II. The nucleic acid content of normal and leukemic nuclei. Cancer Res. 11: 485–489, 1951.

(*17*) HARRINGTON, H., and LAVIK, P. S.: Desoxyribonucleic acid content per nucleus in rat thymus damaged by β-radiation. (Abstract.) Fed. Proc. 10: 194–195, 1951.

(*18*) MITCHELL, J. S.: Disturbance of nucleic acid metabolism produced by therapeutic doses of X- and gamma radiations. II. Accumulation of pentose nucleotides in cytoplasm after irradiation. Brit. J. Exper. Path. 23: 296–309, 1942.

(*19*) GARDELLA, J. W., and LICHTLER, E.: The effect of radiation on the nucleic acid content of the Yoshida ascites tumor cell. (Abstract.) Proc. Am. Assn. Cancer Res. 1: 15–16, 1954.

(*20*) CARNES, W. H., FORKER, B. R., and LIER, E.: Pattern of desoxypentosenucleic acid content of thymic lymphomas induced by X-radiation. (Abstract.) Proc. Am. Assn. Cancer Res. 1: 8–9, 1954.

(*21*) CARNES, W. H., WEISSMAN, N., and GOLDBERG, B.: Desoxyribonucleic acid (DNA) content of tumor cells determined microphotometrically. (Abstract.) Fed. Proc. 11: 410, 1952.

(*22*) SHELTON, E.: Studies on mouse lymphomas. I. Cytology and nucleic acid content. J. Nat. Cancer Inst. 15: 49–61, 1954.

(*23*) PETRAKIS, N. L., and FOLSTAD, L. J.: Desoxyribonucleic acid content of individual lymphoma #1 and #2 tumor cells as determined by Feulgen microspectrophotometry. J. Nat. Cancer Inst. 15: 63–66, 1954.

(*24*) LEUCHTENBERGER, C., KLEIN, G., and KLEIN, E.: The estimation of nucleic acids in individual isolated nuclei of ascites tumors by ultraviolet microspectrophotometry and its comparison with the chemical analysis. Cancer Res. 12: 480–483, 1952.

(*25*) GRIFFIN, A. C., NYE, W. N., NODA, L., and LUCK, J. M.: Tissue proteins and carcinogenesis. J. Biol. Chem. 176: 1225–1235, 1948.

Electron Microscopy of the Virus of Rabbit Fibroma[1]

BOLIVAR J. LLOYD, JR. *and* H. KAHLER,
National Cancer Institute,[2]
Bethesda, Md.

Electron micrographs of myxoma virus have appeared in a number of publications (*1–4*), but heretofore there has been no publication of electron micrographs of the closely related virus of rabbit fibroma. Paschen (*5*) published an optical micrograph of the elementary bodies of fibroma. Because of the close immunological relationship of myxoma to fibroma viruses (*6*), as well as the Berry-Dedrick transformation (*7, 8*), it is of interest to determine whether the fibroma virus resembles the myxoma virus morphologically. For this purpose, the virus of the rabbit fibroma was examined in both the isolated state and in tissue sections.

Materials and Methods

a) *Isolated particles.*—Domestic white rabbits were inoculated with virus derived from material[3] originally isolated by Kilham (*9*) from wild cottontail rabbits trapped in Maryland. After 5 to 15 days the resulting fibromas were harvested. Fibroma tissue, weighing 5 grams was homogenized in a Waring blendor for 5 minutes with 100 ml. of phosphate-citrate buffer, *p*H 7. The resultant suspension was then cleared of tissue debris in a centrifuge operating at 2,000 g for 5 minutes. The procedure described by Dawson and McFarlane (*10*) and Farrant and Fenner (*3*) was then followed. This involved the treatment of the virus for 3 days with 1 *M* NaCl at 4° C., followed by washing in the preparative centrifuge. In the final step the virus was dispersed in solution by exposing it to 9,000 cycles of sonic vibration for 5 minutes. While this procedure was ineffective in purifying fibroma virus to the extent claimed by Dawson and McFarlane, and Farrant and Fenner, for vaccinia and myxoma, it yielded a sufficient number of isolated particles for the purpose of study with the electron microscope.

The final dispersion of fibroma virus was added in small drops to formvar-supporting films. To some of these an equal volume of 2-percent osmium

[1] Received for publication October 14, 1954.
[2] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.
[3] The fibromas were donated by Dr. Lawrence Kilham, whose interest in the characteristics of the virus resulted in this study.

tetroxide solution was added. After drying, the specimens were washed with distilled water and shadowed with chromium.

b) *Tissue sections.*[4]—Small pieces of fibroma from domestic white and wild cottontail rabbits, as well as normal skin tissue from white rabbits, were immersed in cold 1-percent osmium tetroxide solution buffered with veronal-acetate at *p*H 7.2 for 24 hours. The tissues were then dehydrated with alcohols and embedded in a medium consisting of 5 parts *n*-butyl methacrylate and 1 part methyl methacrylate monomer to which 2-percent Luperco was added as a catalyst. Sections were cut with a modified Minot microtome, set to cut at approximately 0.025 microns. The embedding medium was not removed after slicing.

Results

Photographs of isolated particles with no osmic acid are shown in figure 1. These particles have a morphology similar to that described for myxoma virus and vaccinia (*1–3*). The dried particles have a cross section that varies from an irregular circle to a rectangle, usually with rounded corners. Within the particles is a region of higher scattering power than the remainder of the structure. This region is usually centrally located, though in some particles it is near the periphery of the particle.

Photographs of isolated particles treated with osmic tetroxide (fig. 2) indicate that this treatment causes an unevenness of the surface which is not present in the untreated particles.

Measurements on 40 shadowed particles with no osmic fixation gave a size of 269 × 310 mμ, while the average dimensions of 40 particles fixed with osmic acid were 244 × 283 mμ. This is similar to the value 230 × 286 mμ obtained for myxoma by Farrant and Fenner (*3*) on osmic-fixed material.

The shadow lengths for both fixed and unfixed specimens corresponded to a particle height of 110 mμ, which is somewhat higher than the myxoma value of 75 mμ (*3*).

An electron micrograph of a thin slice of fibroma tissue from a cottontail rabbit, harvested 50 days after inoculation (fig. 3), shows the presence of inclusion bodies in the cytoplasm, confirming Fisher's observations (*11*). These bodies appear to be relatively structureless and of various sizes and shapes.

Electron micrographs of younger specimens from a cottontail rabbit (harvested 15 days after inoculation) reveal a high concentration of virus in the cytoplasm (fig. 4). Many of these virus particles give the appearance of having an outer wall of denser structure than the interior, with round to oval cross sections. These particles appear to be similar in size to the isolated particles previously described. On the other hand, virus particles in tissue sections have a more rounded appearance than isolated particles.

[4] These specimens were prepared for us by Dr. A. J. Dalton of this Institute.

Electron micrographs of thin slices of the control tissue (normal skin from a white rabbit) indicate that no virus or inclusion bodies similar to those found in fibroma tissue are present.

Discussion

The preceding results indicate a morphologic similarity in rabbit fibroma virus, rabbit myxoma virus, and the pox viruses. Fibroma virus in tissue sections is ovoid in shape with a "nucleus" and a limiting membrane, while in the isolated state many of the particles have a brick-shaped structure with a dense "nucleus."

The intracytoplasmic inclusions were found in both the domestic and the cottontail rabbits. They showed little or no internal structure and no evidence of virus was present within them. They varied greatly in size, shape, and number present in each cell. However, when many inclusions were present in the cytoplasm, few virus particles were seen.

The fibroma virus in the tissue sections has an appearance similar to that described by Gaylord and Melnick (*12*) for intracellular forms of pox virus.

Summary

1) The rabbit fibroma virus was isolated by differential centrifugation and sonic treatment.

2) Electron micrographs indicated the osmic-fixed preparations had dimensions similar to those of fixed myxoma virus. Nonfixed preparations had dimensions about 10 percent larger than the fixed material.

3) Inclusion bodies were visible in the cytoplasm of sectioned tissue.

4) High concentrations of virus particles in sliced tissue were demonstrable.

References

(*1*) VON BORRIES, B., RUSKA, E., and RUSKA, H.: Bakterien und Virus in übermikroskopischer Aufnahme. Klin. Wchnschr. 17: 921–925, 1938.

(*2*) RUSKA, H., and KAUSCHE, G. A.: Ueber form, grössenverteilung und struktur einiger virus-elementarkörper. Zentralbl. Bakt. (Abt. 1 Orig.) 150: 311–318, 1943.

(*3*) FARRANT, J. L., and FENNER, F.: A comparison of the morphology of vaccinia and myxoma viruses. Australian J. Exper. Biol. & M. Sc. 31: 121–125, 1953.

(*4*) EPSTEIN, B., REISSIG, M. and DE ROBERTIS, E.: Studies by electron microscopy of thin sections of infectious myxomatosis in rabbits. J. Exper. Med. 96: 347–354, 1952.

(*5*) PASCHEN, E.: Ueber das Shopesche infections fibroma of rabbits. Zentralbl. Bakt. (Abt. 1 Orig.) 138: 1–14, 1936.

(*6*) SHOPE, R. E.: Infectious fibroma of rabbits. III. The serial transmission of virus myxomatosum in cottontail rabbits, and cross-immunity tests with the fibroma virus. J. Exper. Med. 63: 33–41, 1936.

(*7*) BERRY, G. P., and DEDRICK, H. M.: A method for changing the virus of rabbit fibroma (Shope) into that of infectious myxomatosis (Sanarelli). J. Bact. 31: 50–51, 1936.

(*8*) ———: Further observations on the transformation of the virus of rabbit fibroma (Shope) into that of infectious myxomatosis (Sanarelli). (Abstract.) J. Bact. 32: 356, 1936.

(*9*) KILHAM, L., and WOKE, P. A.: Laboratory transmission of fibromas (Shope) in cottontail rabbits by means of fleas and mosquitoes. Proc. Soc. Exper. Biol. & Med. 83: 296–301, 1953.

(*10*) DAWSON, I. M., and MCFARLANE, A. S.: Structure of an animal virus. Nature 161: 464–466, 1948.

(*11*) FISHER, E. R.: The nature and staining reactions of the fibroma-cell inclusions of the Shope fibroma of the rabbit. J. Nat. Cancer Inst. 14: 355–363, 1953.

(*12*) GAYLORD, W. H., JR., and MELNICK, J. L.: Intracellular forms of pox viruses as shown by the electron microscope (vaccinia, ectromelia, molluscum contagiosum). J. Exper. Med. 98: 157–172, 1953.

PLATE 58

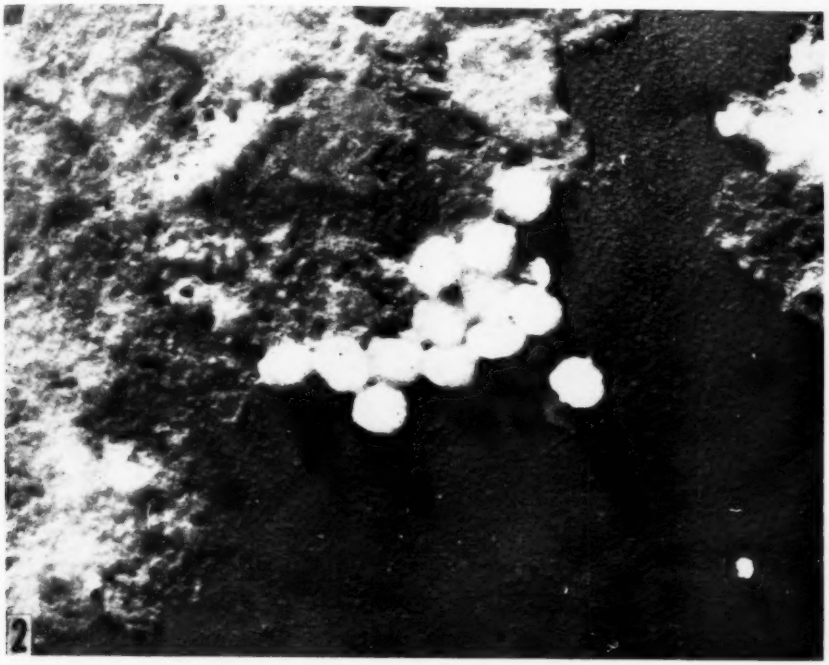
FIGURE 1.—Isolated virus of rabbit fibroma without osmic fixation. Regions of high scattering power within particle can be seen. × 35,000

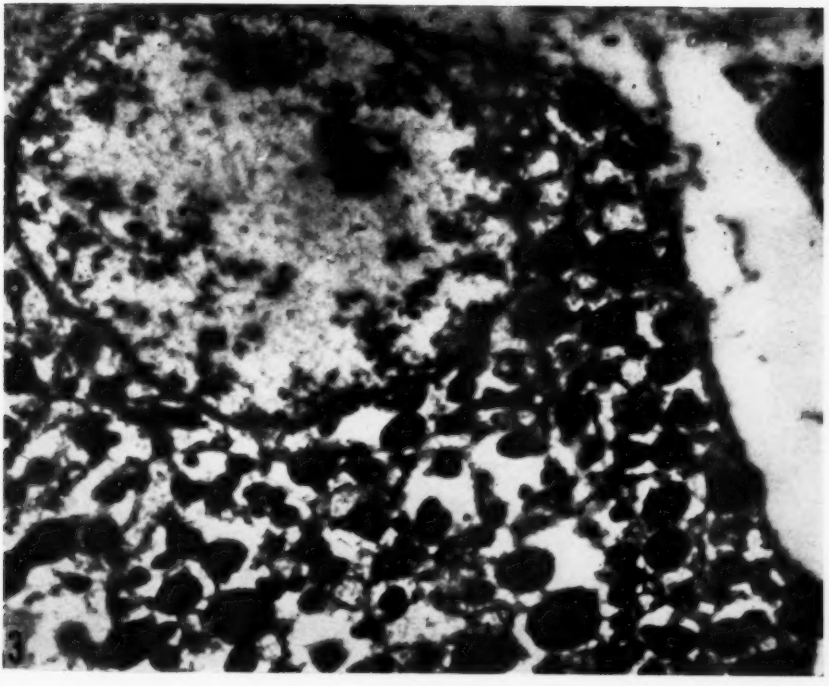
320174—55——16

PLATE 59

FIGURE 2.—A clump of osmic-treated virus particles of rabbit fibroma. Granularity of virus surfaces is visible which is absent in figure 1. × 27,000

FIGURE 3.—Cytoplasmic inclusions of various sizes and shapes in a thin section from a cottontail rabbit. × 8,000

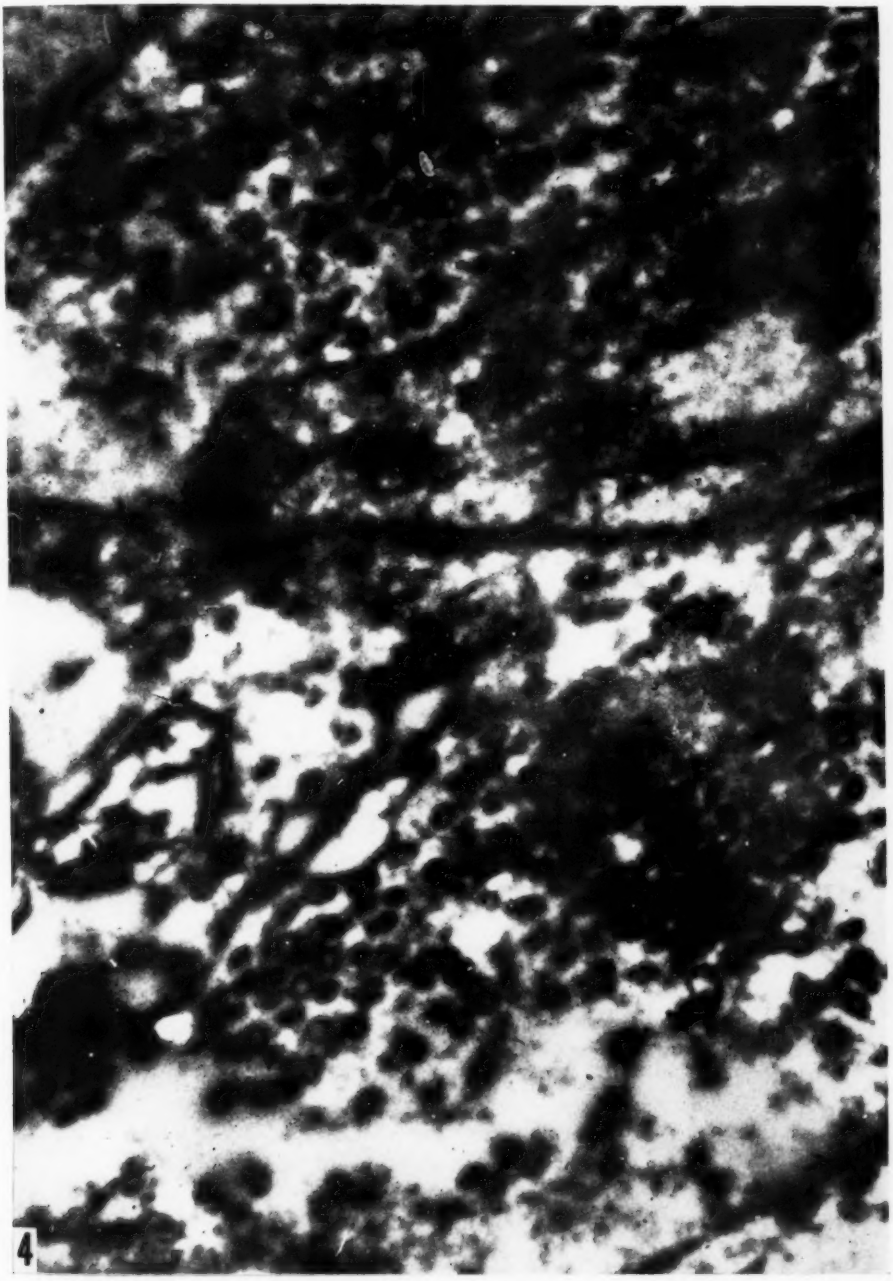




320174—55——17

PLATE 60

FIGURE 4.—Virus particles in the cytoplasm of two adjacent cells. Sectioned virus appears to have a low density interior. × 16,000


4

The *In Vitro* Initiation of Pulmonary Adenomas in Fetal Mouse Lung by a Single Exposure to Ultraviolet Irradiation of Wavelength 2,537 Å[1,2]

STANFIELD ROGERS, *Department of Pathology, Duke University School of Medicine, Durham, N. C.*

The *in vitro* initiation of pulmonary adenomas in mouse lung tissue with methyl bis(β-chloroethyl)amine hydrochloride has been found to have several characteristics in common with the initiation of mutation in the lower organisms (*1*). In *Neurospora crassa*, Kolmark and Westergaard (*2*) have demonstrated that precisely the same mutations may be produced by a single brief exposure to either this nitrogen mustard or to ultraviolet irradiation of a wavelength of 2,537 Å. This region of ultraviolet generally has been found to be that with the greatest mutagenic activity (*3*). In contrast, mixed wavelengths (2,800 to 3,400 Å) have been found the most effective in the production of skin tumors (*4*). This is thought to be, at least in part, the result of the absorption of 2,537 Å of irradiation by the superficial layers of the skin, so that little reaches the proliferating basal layer (*5*) and also in part to the growth-promoting activity of the longer wavelengths (*4*). This latter is particularly pertinent as previous demonstration of the oncogenic effects of ultraviolet radiation has always required repeated exposures over a period of many weeks.

It was determined to find whether a single *in vitro* exposure of mouse lung tissue to ultraviolet radiation of a wavelength of 2,537 Å would suffice to initiate pulmonary adenomas in mouse lung tissue.

Materials, Methods and Results

Strain BALB/c and strain A mice were used. The former were procured originally from the stock of Dr. Peyton Rous of the Rockefeller Institute for Medical Research, New York City, and bred since in this laboratory. Their characteristics have been previously reported upon, both in relation to spontaneous-tumor incidence and the characteristics of the lung tissue upon implantation into other animals of the same strain (*6*). No spontaneous adenomas in the lungs of intact mice have been seen up to at least an age of 6 months. In many implants of this sort no spontaneous tumors

[1] Received for publication September 29, 1954.

[2] This institution was supported by a research grant, C-1741, from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.

have been found on examination in serial section within 10 months of the time of implantation of fetal lung tissue (7). The A strain has been widely used in the study of pulmonary adenomas of mice, and their characteristic of being among the most responsive of mouse strains for the elicitation of pulmonary adenomas with oncogenic agents is well known. A breeding stock of this strain was procured from the Roscoe B. Jackson Memorial Laboratory, Bar Harbor, Maine. The fetal lung tissue used in these experiments was procured and implanted by the general methods outlined by Rous and Smith (8). The ultraviolet light source used was a Mineralight, manufactured by Ultraviolet Products, Inc., South Pasadena, California. According to the manufacturer, 89.7 percent of the light emitted from these units is monochromatic and of a wavelength of 2,537 Å. Less than 3 percent is 3,000 Å and over. The intensity of irradiation of this individual unit at the 5-centimeter distance employed is 1,100 micro watts per square centimeter.

Experiment 1.—Ten strain BALB/c fetuses, measuring 17 to 19 mm. crown-rump and of an estimated 16 to 18 days' gestation, were obtained from two pregnant females. Their lungs were excised, pooled, and hashed between knife blades into fine fragments in Ringer's solution. These hashings varied in size from single cells to lung fragments up to about 1/5 mm. across. After hashing, the lung fragments were divided into two equal parts, placed in separate petri dishes and covered by a film of oxygenated Ringer's solution. This film was less than 1 mm. in thickness. The lung fragments in one of the dishes was then irradiated in a dark room for 15 minutes at 5 cm. from the light source. During the period of irradiation the petri dishes were agitated every 3 to 4 minutes. At the end of the period of exposure the lung fragments from both the irradiated and nonirradiated groups were each suspended in 2 cc. of fresh Ringer's solution. The tissue suspensions were then implanted in opposite posterior thighs of 10 young adult strain BALB/c mice. In each implantation 0.2 cc. of the tissue suspension were used. Two of the implanted mice were discarded early in the experiment because of possible intercurrent disease. Two months after implantation the 8 remaining animals were killed and the implants examined microscopically in serial section for the occurrence of tumors. The results were recorded without knowledge by the investigator of the group from which the implants came.

The tissue grown from the lung hashings exposed to ultraviolet irradiation developed 7 typical pulmonary adenomas, these being distributed among 6 of the 8 implants. No adenomas were found in the implants of the nonirradiated tissue carried in the opposite thigh of the same individuals in which the irradiated tissue was grown.

This finding was confirmed in another strain of mice in the following experiment.

Experiment 2.—Seventeen strain A fetuses were obtained from 2 pregnant females. The mice of one of these litters were 8 in number, measured 12 to 15 mm. crown-rump, and were of an estimated 14 to 15 days' gestation. Those of the other litter were 9 in number, measured 22 to 24 mm.

crown-rump, and were estimated to be at term. Their lungs were excised, pooled, and hashed into fine fragments as before. After hashing, the thoroughly mixed lung fragments were divided into four equal lots which were placed in separate petri dishes and covered with a film of Ringer's solution. This film was less than 1 mm. in thickness. The tissue in three of the petri dishes was irradiated: one for 15, one for 30, and one for 60 minutes. The tissue in the fourth petri dish was not irradiated. The distance from the ultraviolet source was 5 centimeters. All were agitated every 3 minutes. The tissues were then implanted as before into the posterior thigh muscles of 10 young adult strain A mice, the tissue from each individual lot being in one thigh of 5 of these mice. The opposite thigh of these individual mice was implanted with the tissue from another of the lots. Two months after implantation the animals were killed and the implants examined for tumors.

The 5 implants of each tissue irradiated for 15, 30, or 60 minutes contained, respectively, 3, 3, and 2 pulmonary adenomas. These tumors were dispersed singly among the positive implants. No adenomas were found in the implants of tissue which had not been irradiated.

The aggregate amount of tissue growing from the nonirradiated lung implanted was at least twice that of the aggregate of tissue growing from the tissue irradiated for 60 minutes. The aggregate size of the implants of tissue irradiated for 15 and 30 minutes was roughly proportionately intermediate. This manifest lethal effect of increasing the dose of irradiation at the intensity used speaks for effective penetration of the tissues. However, under the conditions of time and intensity used, the variation in the amount of surviving irradiated tissue prohibits interpretation of the result in terms of the relative effect of the duration of irradiation upon the degree of the oncogenic response.

No significance is attached to the difference in responsiveness between the two mouse strains (Experiments 1 and 2), both because of the small number of animals which may be compared and the fact that tissues of strain BALB/c mice grow better on transplantation than do those of strain A animals of the stock used.

Experiment 3.—An experiment similar to Experiment 2, but in strain BALB/c animals, yielded results essentially similar to those of the other two experiments and further confirmed the oncogenic effect of brief *in vitro* exposure to ultraviolet irradiation. Lung hashings from eight 4-day-old, strain BALB/c animals irradiated for 15, 30, and 60 minutes and implanted as usual, yielded respectively, 7, 3, and 4 adenomas. These tumors were distributed among implants carried by 14 mice of strain BALB/c. The aggregate growth of the lung tissue irradiated for 30 and 60 minutes was again considerably less than that irradiated for 15 minutes.

Summary

A single *in vitro* exposure of mouse lung tissue to ultraviolet irradiation of a wavelength of 2,537 Å initiates neoplastic change. Tumors occurred in the exposed lung tissue implanted intramuscularly in mice of the strain

of origin. In the short interval allowed for manifestation of the tumors in implants of the exposed tissue, pulmonary adenomas were the only neoplasms found.

References

(*1*) Rogers, S.: The *in vitro* initiation of neoplastic change with nitrogen mustard. (Abstract.) Sc. Proc. Path. & Bact., Am. J. Path. 29: 607, 1953.

(*2*) Kolmark, G., and Westergaard, M.: Induced back mutations in a specific gene of *Neurospora crassa*. Hereditas 35: 490–506, 1949.

(*3*) Hollaender, A., and Emmons, C. W.: Wavelength dependence of mutation production in the ultraviolet with special emphasis on fungi. Cold Spring Harbor Symp. Quant. Biol. 9: 179, 1941.

(*4*) Rusch, H. P., Kline, B. E., and Baumann, C. A.: Carcinogenesis by ultraviolet rays with reference to wavelength and energy. Arch. Path. 31: 135–136, 1941.

(*5*) Blum, H. F., and Lippincott, S. W.: Carcinogenic effectiveness of ultraviolet radiation of wavelength 2537A. J. Nat. Cancer Inst. 3: 211–216, 1942.

(*6*) Smith, W. E.: The neoplastic potentialities of mouse embryo tissues. V. The tumors elicited with methylcholanthrene from pulmonary epithelium. J. Exper. Med. 91: 87–104, 1950.

(*7*) Rogers, S.: The indirect nature of the action of urethane in initiating pulmonary adenomas in mice. (Abstract.) Fed. Proc. 13: 442–443, 1954.

(*8*) Rous, P., and Smith, W. E.: Neoplastic potentialities of mouse embryo tissues. I. Findings with skin of C strain embryos transplanted to adult animals. J. Exper. Med. 81: 597–620, 1945.

Spontaneous and Radiation-Induced Pituitary Adenomas of Mice[1,2]

ARTHUR C. UPTON *and* JACOB FURTH,[3,4]
Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Tumors of the pituitary gland arise in certain instances when the "hormonal balance" between the hypophysis and its target organs is altered (*1*). The development of such neoplasia after thyroidectomy has been reported to be promoted by ionizing radiation (*2*). Pituitary tumors may also develop after whole-body irradiation alone but have been noted infrequently (*3*) until their occurrence in large numbers of LAF_1 mice irradiated by a nuclear detonation (*4*, *5*). Detailed observations of these tumors form the basis of the present report.

Methods

Male and female LAF_1 [$(C57L \times A)F_1$] mice, 6 to 12 weeks old, were exposed to ionizing radiations from a nuclear detonation. The animals were stationed at various distances from ground zero within thin-walled aluminum chambers that protected them from thermal and mechanical injuries. The radiations were predominantly (over 90%) *gamma* rays, with a smaller component of fast neutrons, the neutron/*gamma* ratio varying with the dose. The doses and numbers of mice exposed are summarized in table 1. Details of the dosimetry will be given in a subsequent report.[5]

Approximately 1 month after irradiation the surviving animals were transported to Oak Ridge. There, individually caged and distributed at random in an air-conditioned room, they were observed until natural death, a few being sacrificed *in extremis*. Post-mortem examinations were performed on all mice, and histologic studies were carried out as required for investigation of special problems or to establish major pathologic diagnoses. All animals were preserved in Kaiserling's solutions, and tissues were fixed in Zenker-formol. Of the more than three thousand

[1] Received for publication October 15, 1954.
[2] Work performed under Contract #W-7405-eng-26 for the Atomic Energy Commission.
[3] *Present address.* Children's Cancer Research Foundation, 35 Binney Street, Boston, Mass.
[4] The authors gratefully acknowledge the technical assistance of W. D. Gude, F. F. Farbstein, E. S. Ledford, and J. R. Thomson.
[5] We are grateful to many members of the Armed Forces and Atomic Energy Commission laboratories for the breeding and irradiation of the mice and their transportation from field laboratories.

mice autopsied, a small number remain to be examined histologically; thus a few pituitary tumors may be discovered on microscopic study in addition to those reported.

Results

Longevity.—Data on the survival of the irradiated mice are summarized in table 1. The LD_{50} per 30 days was approximately 755 r. Shortening of life occurred in proportion to the dose of radiation received, significant reduction of longevity resulting even from the lowest dose studied, *i.e.*, 192 r. Males survived slightly longer than females. Among the major observed causes of death were leukemia, ovarian tumors, mammary gland tumors, and nephrosclerosis, which have been described briefly in a preliminary report (*5*); these bore no discernible relation to the occurrence of pituitary tumors.

TABLE 1.—*Pituitary tumors and longevity in irradiated mice*

Dose (*r*)	Number exposed*	Mean survival (*month*)†		Incidence of pituitary tumors					
				Males			Females		
		♂	♀	Number	Percent‡	Average age at death	Number	Percent‡	Average age at death
						Months			*Months*
812–841	440	19	14	0	0	.....	0	0	.....
759–785	440	19	16	3	5	20	0	0	.....
711–733	440	21	17	2	2	20	15	13	21
631–687	440	23	18	7	4	23	18	12	21
491–556	440	25	22	4	2	22	31	17	24
367–424	440	27	27	1	1	29	10	13	23
287–318	440	28	24	6	3	30	19	9	26
192	220	29	25	1	1	39	7	7	26
0	620	30	28	3	1	37	12	4	31

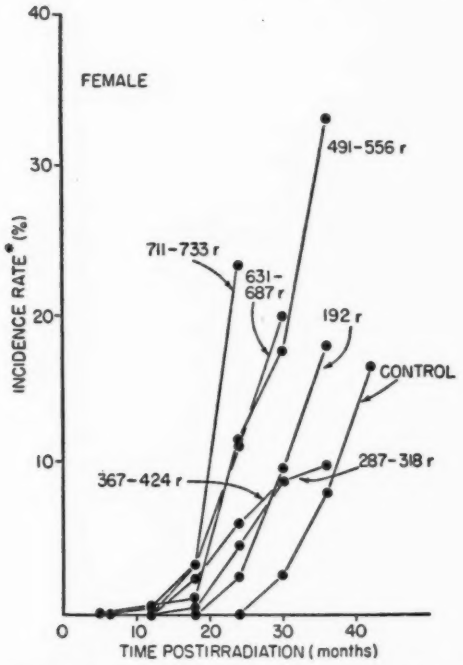
*Approximately half males and half females.

†Longevity of mice received at Oak Ridge; does not include acute (30-day) mortality.

‡Percentage of those surviving one year postirradiation (approximate time of appearance of first pituitary tumors).

Incidence of pituitary tumors.—The frequency of pituitary tumors varied with the dose of radiation (table 1) and was approximately five times greater in females than in males. The neoplasms developed late in life, the earliest appearing 10 months after exposure, and their rate of occurrence increased with age. Pituitary tumors occurred earlier in irradiated mice than in controls, as a function of the dose (text-fig. 1). Tumors were not observed in females exposed to doses above 733 r or in males surviving doses above 785 r, owing probably to the relatively early death of mice exposed to such large amounts of radiation.

Gross observations.—The tumors of the pituitary were spheroid, reddish-brown, soft, and hemorrhagic. When less than 3 mm. in diameter, a tumor usually appeared as a solitary, discrete nodule lateral to the midline; when larger, it tended to fill and erode the sella, obliterating the normal hypophysis and its stalk; indenting and compressing the overlying brain (figs. 1 and 2). [The gross anatomy of the hypophysis of the mouse



TEXT-FIGURE 1.—Pituitary tumors in relation to dose of radiation and time after exposure: * Incidence of mice dying with tumors expressed a percentage of the population surviving.

has been described by Dickie and Woolley (*6*).] Many tumors exceeded 5 mm. in greatest diameter; the largest measured 13 mm. in diameter. There was no correlation between the size of the tumor, and dose of radiation, age at death, or sex.

Miscroscopic observations.—The tumors, stained by Gude's modification (*7*) of the Martins-Mallory trichrome stain (acid fuchsin, aniline blue, orange G, and hematoxylin), were monomorphous and composed of cells predominantly lacking in chromophilic cytoplasmic granules; in most of the neoplasms, however, most tumor cells contained variable numbers of acidophilic granules, those highly granulated resembling the *alpha* cells or acidophils, of the normal gland (fig. 9). Coarse granules which stained pinkish-orange with acid fuchsin and orange G were usually present within the cytoplasm; these were most numerous in the region of the Golgi apparatus and were larger than the more intensely fuchsinophilic granules of the normal *alpha* cells. In no instance were tumor cells observed to contain cytoplasmic granules staining with aniline blue, the periodic acid-Schiff procedure or aldehyde fuchsin. The cells were spheroid to polyhedral and variable in size but uniformly larger than most of the normal cells of the anterior lobe. The negative Golgi image was large and conspicuous, forming a clear ring within the cytoplasm (fig. 9).

The nucleus was large and usually round or ovoid, with several prominent fuchsinophilic nucleoli. Folded, indented, or lobed nuclei were commonly observed. Atypical, hyperchromatic, and giant nuclei were also relatively common (fig. 3), and multinucleate tumor cells were noted occasionally. Mitoses were rare.

The tumor cells were arranged in two major histologic patterns: In solid nests (figs. 4 and 10) or surrounding blood-filled spaces; in the latter case the cell nucleus was oriented toward the basement membrane and the Gogli body toward the cavity (fig. 11). The smaller adenomas exhibited a cystic architecture predominantly, while in the larger, solid tumors this pattern was present only in small foci, usually at the periphery. Congestion and cavernous dilatation of the tumor cysts were observed commonly (figs. 5 and 6), often associated with regional necrosis of the neoplasm, hemorrhage, and hematoma formation. The growths were not encapsulated, and the tumor cells infiltrated adjacent portions of the anterior lobe. Invasion of the pars intermedia, hypophyseal stalk, or brain were rarely noted; however, erosion and infiltration of the sella turcica were seen relatively commonly (fig. 7). The occurrence of multiple adenomata in a single gland was not observed.

Other pituitary changes.—The portions of the anterior hypophysis not invaded by neoplastic cells manifested changes that were also observed in aging controls; these appeared to be accentuated by irradiation and were more marked in females than in males. The *alpha* cells were reduced in size and relative number and their granules appeared somewhat coarser and more sparse than normal. In a small percentage the degranulation was nearly complete. The nuclei of the *alpha* cells were highly irregular in shape, elongated, folded, or lobed (fig. 8), and in some cells atypical division was suggested by such abnormalities as nuclear bridging (fig. 12). Multinuclear cells were common, but mitoses were rarely observed. Hyperchromatic or pyknotic nuclei were often noted. The nucleoli were generally increased in number and enlarged, as was the Golgi apparatus, which usually appeared as a doughnut-shaped clear ring adjacent to the nucleus. *Gamma* cells (chromophobes) appeared to be increased in size and number, and many were vacuolated; there was also enlargement of the nucleus and increase in the size and number of nucleoli. *Beta* and *delta* cells (basophils) were reduced in number. The latter often contained many vacuoles and exhibited prominent and enlarged fuchsinophilic granules, believed to represent mitochondria, in the cytoplasm. Crooke cells were not observed.

Small cysts filled with aniline-blue-staining colloid and lined by flattened cells were noted occasionally in tumor-bearing pituitaries and with about equal frequency in the non-neoplastic hypophyses of aging controls. Vacuolation of the cytoplasm was prominent in the basophils of the pars intermedia in old mice. No abnormalities were observed in the pars nervosa.

Relation of pituitary tumors to changes of other organs.—The incidence of coexisting tumors of other endocrine glands and of the breast is shown in

table 2. Aside from ovarian neoplasms, which were present in more than 60 percent of all irradiated females, tumors of other endocrine or target organs were relatively uncommon and their frequency was slightly if at all affected by pituitary growths.

Hyperplasia of mammary ductal epithelium, alveolar growth, and increased secretory activity were observed in only two male mice with pituitary neoplasia, both of which had adenomas of the adrenal cortex, but such mammary gland changes were common in aging females and greatly exaggerated in those with pituitary tumors. Fibroadenomas, however, were but half as common in animals bearing pituitary tumors.

The thyroid and parathyroid glands appeared normal in all but a few mice; however, the incidence of adenomas of the thyroid was nearly doubled in animals with pituitary tumors.

TABLE 2.—*Changes in endocrine glands and their target organs associated with pituitary tumors*

Organ and effect	Females		Males	
	With pituitary tumor	Without pituitary tumor	With pituitary tumor	Without pituitary tumor
Breast				
Hyperplasia and lactation, degree*	+++	+	0†	0
Fibroadenoma, %‡	0.6	1.3	0	0
Adrenal cortex				
Atrophy, degree	++§	++	++	++
Adenoma, %	7.2	5.2	15.1	11.9
Thyroid gland				
Atrophy, degree	±	±	±	±
Adenoma, %	3.2	1.4	0	0
Submaxillary gland				
Male type, %	37	48	97	96
Uterus				
Atrophy, degree	+++	+++	.....	.....
Seminal vesicle				
Atrophy, degree	.....	.....	±	±
Testis				
Spermatogenesis, degree	.....	.....	++	++
Ovary				
Predominantly luteoma, %	42	51		
Predominantly granulosa-cell tumor, %.	11	9		
Predominantly other type tumor, %.	11	5		

*Average degree of change, 0 to +++.

†Hyperplasia and secretion were present in 2 males with adrenocortical adenomas.

‡Percentage of all tumor-bearing animals and of an equal number of non-tumor-bearing mice of corresponding age, sex, and dose of radiation.

§Slight hyperplasia of zona fasciculata present in 2 mice with ACTH-secreting pituitary tumors (see text).

The adrenal glands of aging mice manifested varying degrees of cortical atrophy, which bore no discernible relation to the presence of pituitary neoplasia; the atrophy, however, developed earlier in the heavily irradiated mice than in controls, corresponding to the earlier appearance of pituitary tumors and to the reduced longevity observed at high dose levels. In both sexes it consisted of a thinning of the zona fasciculata, owing to

decrease in size and number of cells, associated with loss of cytoplasmic neutral fat. In all females there was degeneration of the X zone. In two mice having pituitary tumors that secreted ACTH, as indicated by transplantation and bioassay of the removed neoplasms (*8*), the zona fasciculata was slightly thickened, and its cells were enlarged and finely vacuolated [type *C* cells of Deane *et al.* (*9*)]. Adrenocortical adenomas, composed of large, pale cells similar to those described previously by Woolley and Little as Type "B" cells (*10*), occurred relatively rarely (table 2); they were somewhat more common in males than in females. Their incidence appeared to be slightly greater at high than at low dose levels but could not be correlated with the presence of pituitary growths. Other types of adrenal tumors were not observed.

Gonadal changes were not observed in the male mice, except for moderate testicular atrophy in old age; however, spermatogenesis persisted until death, and no abnormalities of the Leydig cells were noted. In females, ovarian tumors developed in most of the irradiated mice (table 2) irrespective of dose throughout the range studied. These were predominantly of the type designated luteoma on the basis of earlier morphologic and physiologic studies (*11*). Mice bearing luteomas commonly manifested progesterone secretion, as indicated by masculinization of the submaxillary gland (*11*), while those with granulosa-cell tumors usually exhibited evidence of estrogenization in the submaxillary gland and uterus. Also observed were a variety of other types of ovarian tumors similar to those in irradiated mice described previously (*12*). There was no discernible relation between the occurrence of pituitary or adrenal tumors and the gonadal changes just reported. Correspondingly, the uterus did not appear to be influenced by changes of organs other than the ovary; however, hydrometra occurred more commonly in aging nonirradiated females than in those exposed to radiation or bearing pituitary tumors.

Significant changes in other organs were not detected, except for an islet-cell adenoma of microscopic size occurring in a female mouse that received 314 r and died at 20 months of age. The animal had a pituitary tumor which was found on transplantation and bioassay to secrete ACTH (*8*); its adrenals exhibited slight hyperplasia of the zona fasciculata.

Discussion

Relation of pituitary tumors to aging.—The pathogenesis of these pituitary adenomas is not clear. The high-incidence rate of spontaneous neoplasia of this type in aging controls suggests that the tumor-inducing action of radiation may be related to acceleration of a process associated with aging rather than to a specific carcinogenic mechanism. Adenomas of the anterior hypophysis, formerly considered rare, have been recognized in recent years to develop not uncommonly in old age. Thus, Costello found adenomatous proliferation in 225 of 1,000 human pituitaries examined histologically, the incidence being maximal after age 50 (*13*). Similarly, Wolfe *et al.* (*14*) observed pituitary neoplasia to be relatively common in aging rats of two strains. Accordingly, the frequency of hypo-

physeal tumors in the aging mice of this study is not altogether surprising. The paucity of reported spontaneously occurring pituitary neoplasia in mice (*15*, *16*) is attributable in part to the probable existence of genetic differences in predisposition to develop such tumors, as suggested by the varying susceptibility of different strains of mice to induced pituitary neoplasia (*6*, *17*). It is also attributable to insufficient study of the hypophysis in mice of advanced age, the earliest tumors being observed in the nonirradiated females and males of this series at 26 and 33 months of age, respectively.

Classification of tumor cells.—The cells comprising the adenomas were predominantly chromophobic and, to a lesser extent, acidophilic; they differed, however, from normal *alpha* (acidophil) and *gamma* (chromophobe) cells in marked enlargement, hypertrophy of the nucleus, and hypertrophy and hyperplasia of the nucleoli. Coarse, pinkish-orange granules were present in the cytoplasm (staining with acid fuchsin and orange G) similar to those observed in degranulated acidophils of rats treated with estrogen (*18*). The negative Golgi image was enlarged and resembled that observed in degranulated acidophils of aging controls. Similar cells have been observed in pituitary tumors of rats, associated with evidence of lactogenic activity (*19*, *20*), and are distinguishable from the *alpha* cells, or traditional acidophils, by their morphology and staining properties. Such cells have been demonstrated in a variety of species (*21–25*) and are designated by Romeis (*23*) and by Goldberg and Chaikoff (*25*) as *epsilon* cells. Their proliferation in lactating rats suggests that they are the source of prolactin (*24*); an hypothesis consistent with that of Pearse, who believes that both the lactogenic and growth hormones are elaborated by acidophils (*26*). Although it has not yet been possible under ordinary conditions to differentiate tinctorially *epsilon* cells from *alpha* cells in the hypophysis of the mouse, in the pituitary glands of lactating mice proliferation of degranulated acidophils which are cytologically similar to *epsilon* cells has been noted (*27*). Such cells resemble those of the tumors reported above. They have also been observed in aging mice, associated with alterations of the *alpha* cells, such as, degranulation, basophilia of cytoplasm, and hypertrophy of mitochondria and Golgi apparatus. Such changes, commonly interpreted as evidence of heightened secretory activity (*18*), were accompanied by hypertrophy and hyperchromatism of the nucleus and nucleoli, with the frequent occurrence of giant, folded, and lobed nuclei and of multinucleate cells. Similar nuclear alterations in proliferating pituitary cells have been attributed to amitotic cell division or faulty mitosis (*6*, *26*); the latter explanation is favored by the observation of nuclear bridging (fig. 12).

Gonadal factors in pituitary tumorigenesis.—Morphologically, the pituitary adenomas observed in this study are similar to some of the tumors noted in rats after prolonged administration of estrogen (*19*, *20*) and in old age (*14*, *20*). The pathogenesis of estrogen-induced pituitary tumors has been reviewed by Gardner (*1*) and by Bielschowsky (*20*), who report that in some instances the tumors behave as "conditioned neoplasms"

(*29*) undergoing regression upon withdrawal of the estrogenic stimulus. That the tumors in the present study were induced by endogenous estrogen would seem doubtful in the light of the appearance of secondary sexual organs. In males, with or without pituitary tumors, the seminal vesicles were usually only slightly atrophic and seldom distended; in females the uterus was almost always atrophic, except in the presence of a functionally active ovarian tumor. The submaxillary glands were masculinized in about one-third of the tumor-bearing females (attributable to coexisting luteomas) and in all the tumor-bearing males examined except one which had a coexisting adrenocortical adenoma. Non-tumor-bearing animals of corresponding age presented comparable findings.

The occurrence of pituitary tumors in old females, *i.e.*, after the climacteric, and after sterilizing doses of ionizing radiations suggests that the neoplasia might have been induced by hypogonadism. Nevertheless, there is no anatomic evidence that the pituitary tumors were gonadotrophic or were induced by an imbalance of gonadal hormones. The development of pituitary tumors in males certainly cannot be attributed to castration. The fertility of male mice is only temporarily depressed after doses of irradiation of the type studied herein, and male mice are known to remain fertile into old age. Spermatogenesis was observed in tumor-bearing males without exception, and the Leydig cells appeared well preserved until death.

Evidence of secretion of gonadotrophins was not manifested by mice bearing grafts of tumors induced by irradiation (*8*). Furthermore, the pituitary tumors which have been reported to develop in mice after gonadectomy (*6*) failed to attain great size and were described as being composed of basophilic cells, thus differing from those of the present experiment; however, both types of tumors exhibited lactogenic activity.

Thyroid factors in tumorigenesis.—That the observed pituitary tumors were not induced by thyroidectomy is evident from the predominantly normal thyroid status of the mice of this study, as indicated in histologic sections, and from differences in the morphologic features of the anterior pituitary in the animals of the present series as compared with those subjected to thyroidectomy (*30–33*). The changes in *beta* cells reported in the latter (*31–33*) were not observed in this study. Furthermore, the tumor cells described above, while amphophilic in the strict sense of the word, were morphologically and cytochemically unlike those of thyroidectomy-induced tumors (*33*). Thus, after fixation in Zenker-formol none were observed to contain aniline blue-staining granules or to react positively with either the periodic acid-Schiff or the aldehyde-fuchsin techniques.

The higher incidence of thyroid adenomas (table 2) in mice with pituitary tumors than in those without pituitary tumors is of borderline significance ($P=0.20$) but suggests that at least a few of the hypophyseal tumors exerted a thyrotrophic action. This was borne out on transplantation and bioassay, one of the seven adenomas successfully transplanted

exhibiting predominantly thyroid-stimulating and somatotrophic activity (*34*).

The slightly greater incidence of thyroid adenomas in females, as compared with males (table 2), may be correlated with the greater susceptibility of female mice to the induction of thyrotrophic pituitary tumors and thyroid changes (*35*). Although whole-body ionizing irradiation has been reported by Gorbman and Edelmann to promote the development of pituitary tumors in mice given low doses of radioiodine (*2*), others believe that radiation plays no significant role in the induction of these highly thyrotrophic tumors (*35*).

Adrenal factors in tumorigenesis.—The production of pituitary tumors by adrenalectomy has not been reported, but thus far adrenalectomized animals have not been kept alive long enough for the study of pituitary tumor-formation. Despite evidence that three of the ten assayed adenomas of these mice yielded transplantable tumors secreting ACTH (*8*), it remains to be shown that the development of the pituitary neoplasia was dependent upon altered adrenal function, because the adrenal glands of the tumor-bearing hosts were not significantly different from those of tumor-free controls. The effects of irradiation and aging on adrenal function remain to be critically examined.

Although adrenocortical adenomas occurred slightly more often in males than in females (table 2), the difference was not highly significant ($P=0.23$). A female predilection for adrenocortical tumors was observed in gonadectomized mice (*6*); such adenomas, morphologically similar to those observed in this study, were believed to secrete estrogen (*6*). Similarly, in the present study a male mouse with an adrenocortical adenoma exhibited estrogenization of the submaxillary glands and mammary gland hyperplasia.

The slightly greater incidence ($P=0.34$) of adrenal tumors in the mice of both sexes bearing pituitary growths (table 2) recalls the observation of ACTH secretion by three of the seven hypophyseal adenomas that were successfully transplanted (*8*). These three grafted tumors exhibited adrenocorticotrophic potency in hypophysectomized hosts but failed to sustain other endocrine target organs (*34*).

Secretory activity in relation to cell type.—The mammary gland stimulation noted uniformly in females bearing primary pituitary tumors was exhibited on transplantation only in female hosts and with but three of the seven adenomas assayed; with one tumor it was the sole effect detected (*34*). The evidence of secretion of adrenocorticotrophin, thyrotrophin, and sonatotrophin by some of the other grafted tumors indicates that the adenomas of this group are heterogeneous in their secretory activity. This is difficult to reconcile with the observation that the primary tumors were composed exclusively of chromophobic and acidophilic cells. For although, as noted above, the lactogenic and growth hormones are believed to be produced by acidophils (*26*), it is thought that the basophils are the source of TSH (*36*) and ACTH (*37*). Elucidation of this question will require further investigation.

Summary

Tumors of the adenohypophysis developed spontaneously in LAF_1 mice, appearing first at about two years of age and occurring in more than 8 percent of females surviving three years.

The incidence of such tumors was increased by a single whole-body exposure to ionizing radiation, and tumor-formation was hastened in proportion to the dose.

The neoplasms were four to five times more common in females than in males.

The adenomas were composed of cells predominantly chromophobic although containing in the cytoplasm varying numbers of coarse acidophilic granules; most neoplasms also contained at least a few heavily granulated acidophils which were interpreted as tumor cells.

The pituitary-tumor-bearing females exhibited alveolar hyperplasia and distension of mammary gland ducts, which were also occasionally present to a lesser degree in aging females without pituitary tumors.

References

(*1*) GARDNER, W. U.: Hormonal imbalances in tumorigenesis. Cancer Res. 8: 397–411, 1948.

(*2*) GORBMAN, A. and EDELMANN, A.: Role of ionizing radiation in eliciting tumors of the pituitary gland in mice. Proc. Soc. Exper. Biol. & Med. 81: 348–350, 1952.

(*3*) FURTH, J., and BUTTERWORTH, J. S.: Neoplastic diseases occurring among mice subjected to general irradiation with x-rays. II. Ovarian tumor and associated lesions. Am. J. Cancer 28: 66–95, 1936.

(*4*) UPTON, A. C., and FURTH, J.: Induction of pituitary tumors by means of ionizing irradiation. Proc. Soc. Exper. Biol. & Med. 84: 255–257, 1953.

(*5*) FURTH, J., UPTON, A. C., CHRISTENBERRY, K. W., BENEDICT, W. H., and MOSHMAN, J.: Some late effects in mice of ionizing radiation from an experimental nuclear detonation. Radiology 63: 562–569, 1954.

(*6*) DICKIE, M. M., and WOOLLEY, G. W.: Spontaneous basophilic tumors of the pituitary glands in gonadectomized mice. Cancer Res. 9: 372–384, 1949.

(*7*) GUDE, W. D.: Modified Martins-Mallory stain for mouse pituitary gland. Stain Technol. 28: 161–162, 1953.

(*8*) FURTH, J., GADSEN, E. L., and UPTON, A. C.: ACTH secreting transplantable pituitary tumors. Proc. Soc. Exper. Biol. & Med. 84: 253–254, 1953.

(*9*) DEANE, H. W., SHAW, J. H., and GREEP, R. O.: The effect of altered sodium or potassium intake on the width and cytochemistry of the zona glomerulosa of the rat's adrenal cortex. Endocrinology 43: 133–153, 1948.

(*10*) WOOLLEY, G. W., and LITTLE, C. C.: The incidence of adrenal cortical carcinoma in gonadectomized female mice of the extreme dilution strain. I. Observations on the adrenal cortex. Cancer Res. 5: 193–202, 1945.

(*11*) FURTH, J., and SOBEL, H.: Transplantable luteoma in mice and associated secondary changes. Cancer Res. 7: 246–262, 1947.

(*12*) BALI, T., and FURTH, J.: Morphological and biological characteristics of X-ray induced transplantable ovarian tumors. Cancer Res. 9: 449–472, 1949.

(*13*) COSTELLO, R. T.: Subclinical adenoma of the pituitary gland. Am. J. Path. 12: 205–216, 1936.

(*14*) WOLFE, J. M., BRYAN, W. R., and WRIGHT, A. W.: Histologic observations on the anterior pituitaries of old rats with particular reference to the spontaneous appearance of pituitary adenomata. Am. J. Cancer 34: 352–372, 1938.

(*15*) SLYE, M., HOLMES, H. F., and WELLS, H. G.: Intracranial neoplasms in lower animals; studies in the incidence and inheritability of spontaneous tumors in mice. Am. J. Cancer 15: 1387–1400, 1931.

(*16*) GARDNER, W. U., STRONG, L. C., and SMITH, G. M.: An observation of primary tumors of the pituitary, ovaries, and mammary glands in a mouse. Am. J. Cancer 26: 541–546, 1936.

(*17*) GARDNER, W. U., and STRONG, L. C.: Strain-limited development of tumors of the pituitary gland in mice receiving estrogens. Yale J. Biol. & Med. 12: 543–548, 1940.

(*18*) WOLFE, J. M.: Cytochemical studies of the anterior hypophyses of rats receiving estrogen. Am. J. Anat. 85: 309–345, 1949.

(*19*) WOLFE, J. M., and WRIGHT, A. W.: Histologic effects induced in the anterior pituitary of the rat by prolonged injection of estrin with particular reference to the production of pituitary adenomata. Endocrinology 23: 200–210, 1938.

(*20*) BIELSCHOWSKY, F.: Functional acidophilic tumours of the pituitary of the rat. Brit. J. Cancer 8: 154–160, 1954.

(*21*) CLEVELAND, R., and WOLFE, J. M.: A differential stain for the anterior lobe of the hypophysis. Anat. Rec. 51: 409–413, 1932.

(*22*) DAWSON, A. B., and FRIEDGOOD, H. B.: Differentiation of two classes of acidophiles in the anterior pituitary of the female rabbit and cat. Stain Technol. 13: 17–21, 1938.

(*23*) ROMEIS, B.: Handbuch der Mikroskopischen Anatomie der Menschin. Berlin, Springer, 1940, vol. 6, part 3.

(*24*) LACOUR, F.: Recherches sur la relation entre les cellules hypophysaires à granulations orangées (cellules e de Romeis) et les phénomènes de lactation. Compt. rend Soc. biol. 144: 248–249, 1950.

(*25*) GOLDBERG, R. C., and CHAIKOFF, I. L.: On the occurrence of six cell types in dog anterior pituitary. Anat. Rec. 112: 265–274, 1952.

(*26*) PEARSE, A. G. E.: Cytochemical localization of the protein hormones of the anterior hypophysis. *In* Ciba Foundation Colloquia on Endocrinology. New York, The Blakiston Co., 1952, vol. 4, pp. 1–20.

(*27*) UPTON, A. C., and GUDE, W. D. Unpublished data.

(*28*) SELYE, H.: Atypical cell proliferation in the anterior lobe adenomas of estradiol-treated rats. Cancer Res. 4: 349–351, 1944.

(*29*) FURTH, J.: Conditioned and autonomous neoplasms: *A review.* Cancer Res. 13: 477–492, 1953.

(*30*) HALMI, N. S., and GUDE, W. D.: The morphogenesis of pituitary tumors induced by radiothyroidectomy in the mouse and the effects of their transplantation on the pituitary body of the host. Am. J. Path. 30: 403–419, 1954.

(*31*) GORBMAN, A.: Tumorous growths in the pituitary and trachae following radiotoxic dosages of I^{131}. Proc. Soc. Exper. Biol. & Med. 71: 237–240, 1949.

(*32*) FURTH, J.: Morphologic changes associated with thyrotrophin-secreting pituitary tumors. Am. J. Path. 30: 421–463, 1954.

(*33*) BURT, A. S., LANDING, B. H., and SOMMERS, S. C.: Amphophil tumors of the hypophysis induced in mice by I^{131}. Cancer Res. 14: 497–502, 1954.

(*34*) FURTH, J., ANDERSON, E.. and GADSEN, E. L.: Unpublished data.

(*35*) DENT, J. N., GADSEN, E. L., and FURTH, J.: On the relation between thyroid depresssion and pituitary tumor induction in mice. Cancer Res. In press.

(*36*) PURVES, H. D., and GRIESBACH, W. E.: The site of thyrotrophin and gonadotrophin production in the rat pituitary studied by McManus-Hotchkiss staining for glycoprotein. Endocrinology 49: 244–264, 1951.

(*37*) MARSHALL, J. M., JR.: Localization of adrenocorticotropic hormone by histochemical and immunochemical methods. J. Exper. Med. 94: 21–30, 1951.

PLATE 61

FIGURE 1.—Medium-sized pituitary tumor. × 3

FIGURE 2.—Large pituitary tumor filling base of skull. × 3

FIGURE 3.—Pituitary tumor cells with atypical and giant nuclei. Hematoxylin and eosin. × 440

FIGURE 4.—Solid adenoma replacing right lobe of anterior pituitary gland (*bottom*). The tumor cells are predominantly chromophobic and larger than those of the non-neoplastic, left lobe (*left*). Pars intermedia and pars nervosa are shown at the top. Gude-Martins-Mallory. × 120

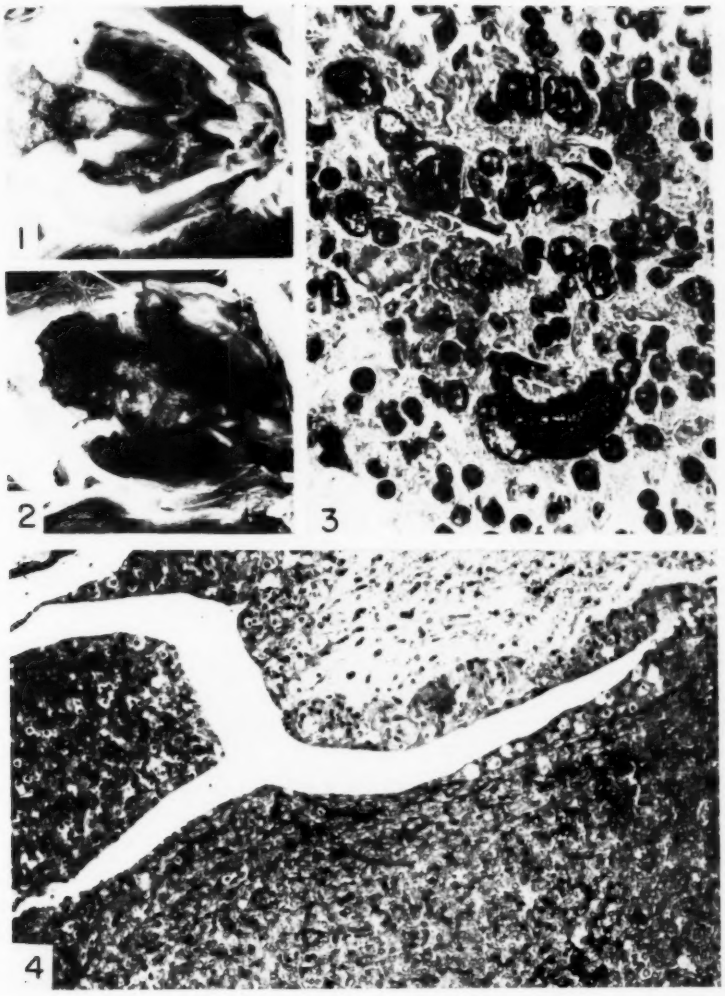

1
2
3
4

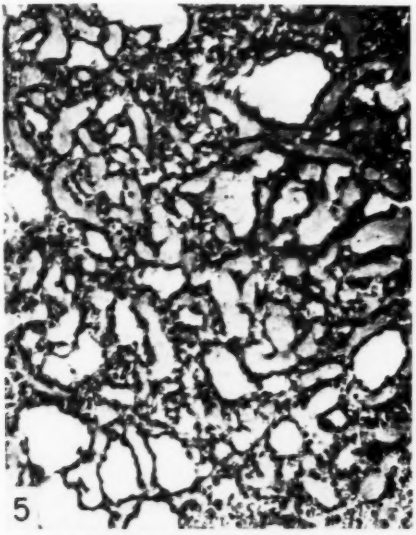
PLATE 62

FIGURE 5.—Adenoma with markedly dilated blood-filled cysts. Hematoxylin and eosin. × 120

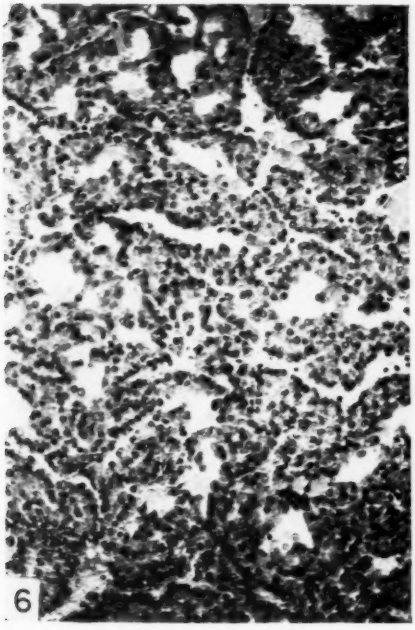
FIGURE 6.—Tumor cells arranged around cysts. Gude-Martins-Mallory. × 440

FIGURE 7.—Nest of metastatic tumor cells in marrow of sella turcica. Pituitary tumor shown at top. Hematoxylin and eosin. × 120

FIGURE 8.—Acidophiles (dark-staining cytoplasm) of aging control mouse exhibiting degranulation and pleomorphism of nuclei. Gude-Martins-Mallory. × 1,000


5

7


6

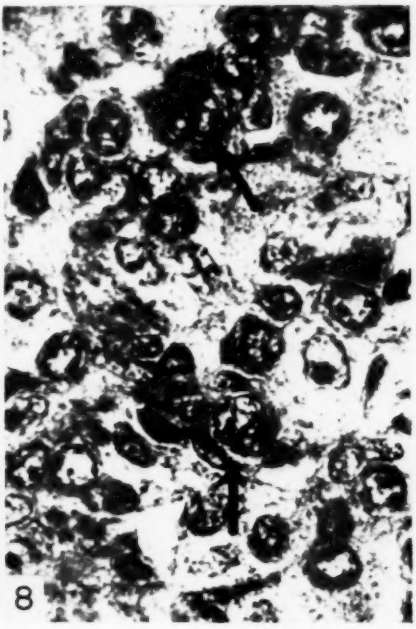

8

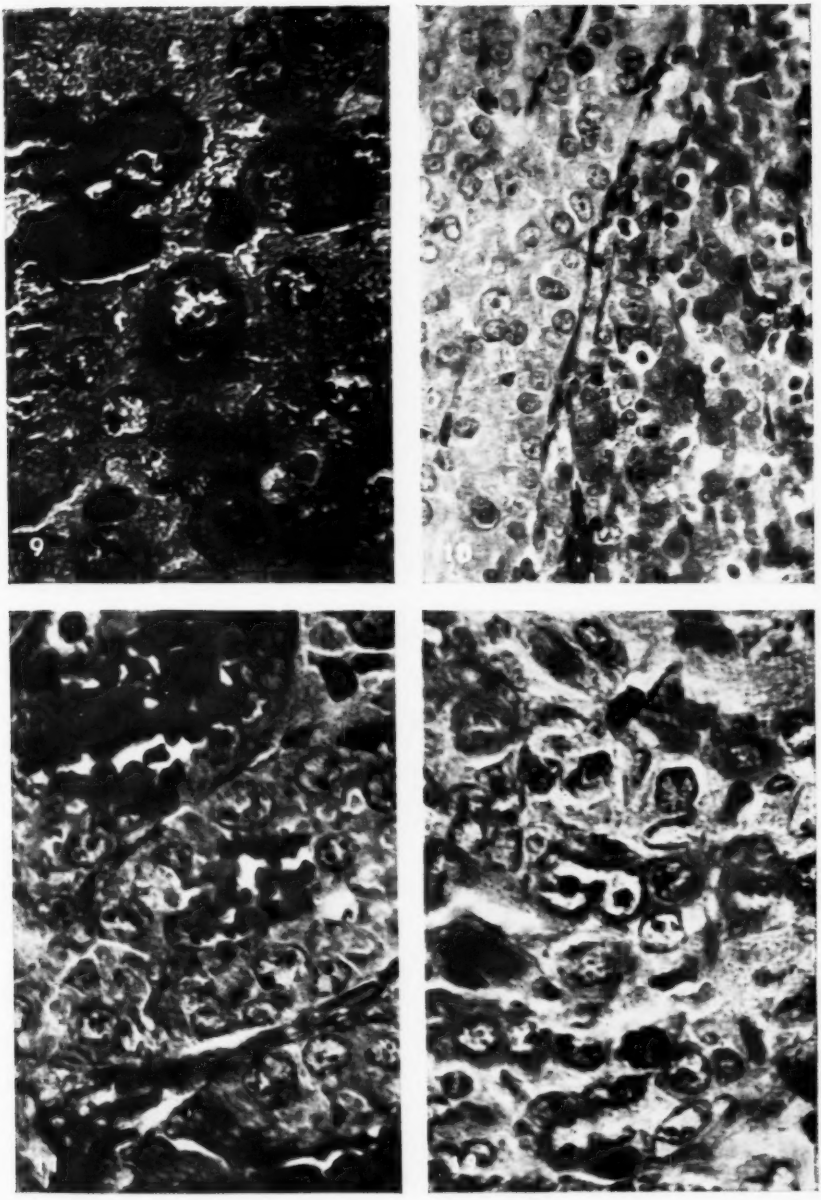
PLATE 63

FIGURE 9.—Pituitary tumor cells most of which contain varying numbers of acidophilic cytoplasmic granules and prominent ring-shaped negative Golgi images. Gude-Martins-Mallory. × 1,000

FIGURE 10.—Boundary between solid chromophobic adenoma (*left*) and normal anterior pituitary (*right*). Gude-Martins-Mallory. × 440

FIGURE 11.—Predominantly chromophobic tumor cells encircling blood-filled spaces. Nuclei oriented toward basement membrane and Golgi body toward cavity. Gude-Martins-Mallory. × 1,000

FIGURE 12.—Acidophil of aging control mouse exhibiting nuclear bridging. Gude-Martins-Mallory. × 1,000


9

Successful Skin Homografts After the Administration of High Dosage X Radiation and Homologous Bone Marrow[1]

JOAN MICHEL MAIN *and* RICHMOND T. PREHN,[2]
National Cancer Institute,[3] Bethesda, Md.

The known factors determining tissue-graft survival may be conveniently classified under two headings: 1) the genetic and 2) the environmental. Early workers with cancer, who searched for a method of transplanting tumors from one animal to another, began the study which led to the elucidation of the basic genetic principles of tissue transplantation. These principles, which explain tissue-graft resistance in terms of Mendel's laws of heredity, have been thoroughly reviewed (*1*, *2*). Briefly, they may be summarized as follows: *a*) the inheritance of tissue-graft susceptibility is due to certain multiple dominant genes, called histocompatibility genes; and *b*) grafts tend to survive if the recipient contains all (or even additional) histocompatibility genes present in the donor. With the development of inbred strains of animals (*i.e.*, homozygous for practically all genes), this condition could more nearly be met, and tumors and normal tissues (where no technical difficulties exist) are now transplanted freely among the different individuals of a single inbred strain. An increasing amount of evidence indicates that the resistance to genetically dissimilar tissue may be an as yet undetermined type of immune reaction (*3–7*).

Environmental factors often alter resistance to homografts[4] by weakening the immune response of the host. These factors include vital staining (*8*, *9*), application of hormones (*10*), and X irradiation of the host (*11*). Dempster, Lennox and Boag (*12*) were able to prolong significantly the survival time of homologous skin grafts in rabbits by administering high dosages of X radiation prior to grafting.

Although X irradiation has been shown to prolong the survival time of homologous skin grafts, the life of the host is shortened as the dosage of X radiation increases. Recently, Lorenz, Congdon and Uphoff (*13*) have increased the 30-day survival time of mice given an LD_{100} dosage of X radiation by the injection of isologous, homologous and, in some cases,

[1] Received for publication October 25, 1954.
[2] With the technical assistance of Earl J. Waters.
[3] National Institutes of Health, U. S. Public Health Service, Department of Health, Education, and Welfare.
[4] Definition of terms: *heterologous graft:* a transplant between animals of different species; *homologous graft:* a transplant between animals of the same species but of different genotypes; *isologous graft:* a transplant between animals of the same genotype.

320174—55——19

heterologous bone marrow immediately following exposure to X radiation. In the following experiments an attempt was made to prolong the survival time of homologous skin grafts by treating the graft recipient with high doses of X radiation followed by bone-marrow injection.

Materials and Methods

The method of X irradiation was as follows: To obtain a uniform tissue dose, two X-ray tubes were placed opposite each other, above and below the box containing the mice. The distance from the targets to the center of the mice was 54 cm. The tubes operated at 200 kv. and 15 Ma. The beams were filtered by plates of 0.25 mm. thick copper and 0.55 mm. thick aluminum. A flat circular lucite box measuring six inches in diameter and one inch in depth, capable of accommodating 8 mice, was used as a container for the mice during administration of the X radiation.[5]

The bone marrow was prepared according to the method of Lorenz *et al.* (*13*), with the exception that physiological saline was substituted for buffered saline. In all of these experiments marrow from both humeri and both femura of two donor animals was used for each recipient and the marrow was injected into a tail vein.

The method of skin grafting has been previously published (*14*). To summarize, a full-thickness graft was transferred from the back of the donor to that of the recipient. The donor mice were killed by cervical dislocation immediately before grafting and the recipients were anesthetized with nembutal anethesia.

Strain BALB/cAnN, strain DBA/2JN, and (BALB/cAnN × DBA/2)F_1 hybrid mice were used in these experiments and were obtained from the inbred mouse colonies at the National Institutes of Health.

Strain DBA/2JN female mice 2 to 8 months of age were divided into three groups. The mice of all three groups were grafted 24 to 30 days after X irradiation with skin from male BALB/cAnN mice 2 months of age.

Group I.—These mice were irradiated with a dosage of 350 r. They were not injected with bone marrow.

Group II.—These mice were irradiated with a dosage of 750 to 800 r[6] and were then immediately injected intravenously with strain DBA/2JN bone marrow. In this case the injected marrow was isologous to the recipient but homologous to the skin grafts.

Group III.—These mice were irradiated with a dosage of 750 to 800 r and were then immediately injected intravenously with (BALB/cAnN × DBA/2JN)F_1 hybrid bone marrow. In this case the injected marrow was homologous to the recipient but contained an antigenic component identical with the antigens of the skin grafts.

[5] The authors wish to express their appreciation to Mr. Henry Meyer, who X-irradiated the animals in these experiments.

[6] In view of the X-irradiation deaths in group I and the X-irradiation tolerances which have been determined for other strains, it is thought that the mice of groups II and III would not have survived in the absence of bone-marrow injections. In addition, Reinhard (*15*) has found 528 r to be the LD_{30} at only 12 days for 8- to 15-week-old DBA mice of either sex.

Heterozygous (BALB/cAnN × DBA/2JN)F_1 hybrid marrow cells were used in preference to homozygous BALB/cAnN cells for two reasons: First, the hybrid cells might stimulate less of an immune reaction in the recipient than would homologous homozygous marrow cells. [Prehn and Main (*16*) have shown that (BALB/cAnN × DBA/2JN)F_1 hybrid skin survives for a significantly longer time than does homozygous BALB/cAnN skin when grafted onto DBA/2JN mice.] Second, bone marrow, even though transplanted, might function to produce antibodies against suitable antigens. Hybrid bone marrow was used in this experiment because, according to the principles of transplantation, it was not expected to be antigenically stimulated by either the strain DBA/2JN tissues of the recipient mice or by the BALB/cAnN tissues of the skin grafts.

Results

The detailed data of these experiments are shown in table 1.

In previous skin-grafting experiments done in this laboratory (*16*) between strains BALB/cAnN and DBA/2JN (BALB/cAnN skin on DBA/2JN mice) no successful grafts were obtained in a total of 61 animals. The average graft-survival time was approximately 30 days. All grafts had sloughed by the 45th day. To date, our experience with skin-grafting experiments has been that even very slight genetic differences, such as are found between sublines of the same strain, are usually enough to prevent successful grafting.

Group I.—None of the DBA/2JN mice that received sublethal dosages of 350 r of X radiation retained their grafts of BALB/cAnN skin significantly longer than did the nonirradiated mice. The average graft-survival time in this group was about 32 days. All had sloughed their grafts by the 45th day.[7] This result differs from that reported by Dempster *et al.* (*12*), who were able to prolong significantly the survival time of homologous grafts in rabbits by X irradiation prior to grafting.

Group II.—Mice that received high dosages of X radiation (750–800 r) and bone marrow *homologous to the graft* (isologous to the recipient) varied in their graft responses. In this group most mice retained their grafts longer than mice in Group I. However, only 2 mice in 31 (7%) retained their grafts permanently. The average graft-survival time, excluding the two permanent survivals, was approximately 50 days.

Group III.—Almost all mice that received high doses of radiation (750–800 r) followed by hybrid bone marrow were grafted successfully. In this group, 33 of 36 (92%) grafted mice retained their grafts. These grafts appeared as healthy as autografts (see fig. 1). The difference between group II and III was statistically significant, using the chi-square method of analysis ($P<.01$).

Discussion

The precise mechanism of action of the bone marrow in these experiments remains obscure. Probably, the bone marrow allowed an unusually

[7] The criteria by which the graft-survival time was judged were the same as those previously described (*16*).

TABLE 1.—*Graft-survival time for mice that received: no marrow; isologous marrow; or homologous F_1 hybrid marrow following X irradiation**

Group	Mice (*number*)	X radiation dosage (*r*)	Mice surviving irradiation (*percent*)	Successful grafts (*percent*)	Graft-survival time (*Successful grafts*) †
I (no marrow)	15	350	87. 5	none	
II (DBA/2JN marrow)	37	750–800	84. 0	7 (2 in 31)	1 mouse—180 days (to date) 1 mouse—60 days (to date)
III [(BALB/cAnN × DBA/2JN) F_1 hybrid marrow].	39	750–800	92. 0	92 (33 in 36)	4 mice—180 days (to date) 8 mice—150 days (to date) 9 mice—90 days (to date) 12 mice—60 days (to date)

*All host mice used were DBA/2JN females, 2–8 months of age. All skin donors were BALB/cAnN males, 2 months of age. Elapsed time between irradiation and grafting was 24–30 days.
†Average survival time of unsuccessful grafts was approximately 32 days in Group I and 50 days in Group II.

large dosage of X radiation to be employed. But, in addition, the genetic composition of the marrow was a factor determining graft results. Thus, most of the mice that were injected with marrow containing an antigenic component presumably identical with the antigens of the graft retained their grafts. On the other hand, most of those receiving marrow *not* containing an antigenic component presumably identical with the antigens of the graft sloughed their grafts. Therefore, it may be concluded that the bone marrow served some function in addition to permitting host survival.

Recently, a preliminary report concerning a phenomenon which may act by a mechanism similar to the one described in this paper has been published by Billingham, Brent, and Medawar (*17*). These authors found that by inoculating embryonic chicks or mice with homologous living cells they could produce a partial or complete tolerance to skin grafts subsequently transplanted from the animal that provided the inoculum.

Perhaps the marrow homologous to the host served merely as a source of homologous antigen during the period of maximal radiation effect. Possibly the immunologic response of the host after exposure to the high doses of X radiation used here was so weak that any homologous graft, *whether bone marrow or skin*, performed immediately after X irradiation would have survived and have permanently altered the host's reaction. Should the injected homologous marrow cells and their descendants survive permanently, *i.e.*, throughout the remaining life span of the host, the resultant animal would be a "pseudohybrid," genetically constituted DBA/2JN but with its bone marrow partially derived from (BALB/cAnN×DBA/2JN)F_1 hybrid cells. The transplanted (BALB/cAnN×DBA/2JN)F_1 hybrid marrow would not be capable of immunologic response to further grafts containing BALB/cAnN antigens.

Since there is no reason to assume that the host would offer greater resistance to a bone-marrow graft than to a skin graft, the results of this experiment are consistent with the *cellular repopulation* theory of radiation protection (*13*, *18*).

For obvious reasons, much further work will be required before one could possibly expect a general solution to the homograft problem.

Conclusions

1) Strain BALB/cAnN skin may not be successfully grafted onto strain DBA/2JN female mice if the host mouse is treated with an X-radiation dosage of 350 r and grafted 24 to 30 days after exposure.

2) Strain BALB/cAnN skin may not usually be grafted successfully onto strain DBA/2JN female mice if the host animal is treated with an X-radiation dosage of 750 to 800 r followed by the immediate intravenous injection of strain DBA/2JN bone marrow 24 to 30 days prior to grafting.

3) Strain BALB/cAnN skin may be successfully grafted onto strain DBA/2JN female mice if the host animal is treated with an X-radiation dosage of 750 to 800 r followed by the immediate intravenous injection of (BALB/cAnN × DBA/2JN)F_1 hybrid bone marrow 24 to 30 days prior to grafting.

References

(1) LITTLE, C. C.: The genetics of tumor transplantation. *In* The Biology of the Laboratory Mouse (Snell, G. D., ed.). Philadelphia, The Blakiston Co., 1941, pp. 279–309.

(2) SNELL, G. D.: The genetics of transplantation. J. Nat. Cancer Inst. 14: 691–700, 1953.

(3) PREHN, R. T., and MAIN, J. M.: Mobility and site of production of homograft resistance factor. J. Nat. Cancer Inst. 14: 1277–1280, 1953.

(4) MEDAWAR, P. B.: A second study of the behaviour and fate of skin homograft on rabbits. J. Anat. 79: 157–176, 1945.

(5) PREHN, R. T., WEAVER, J. M., and ALGIRE, G. H.; The diffusion-chamber technique applied to a study of the nature of homograft resistance. J. Nat. Cancer Inst. 15: 509–517, 1954.

(6) ALGIRE, G. H., WEAVER, J. M., and PREHN, R. T.: Growth of cells *in vivo* in diffusion chambers. I. Survival of homografts in immunized mice. J. Nat. Cancer Inst. 15: 493–507, 1954.

(7) BARRETT, M. K., and HANSEN, W. H.: Resistance to tumor implantation induced by red cell stromata. Cancer Res. 13: 269–275, 1953.

(8) LUDFORD, R. J.: Resistance to growth of transplantable tumours. I. Influence of vital staining on induced resistance. Brit. J. Exper. Path. 12: 45–49, 1931.

(9) ———: Resistance to the growth of transplantable tumours. II. Influence of dyestuffs and colloids on natural resistance. Brit. J. Exper. Path. 12: 108–113, 1931.

(10) BILLINGHAM, R., KROHN, P., and MEDAWAR, P.: Effect of cortisone on survival of skin homografts in rabbits. Brit. Med. J. 1: 1157–1163, 1951.

(11) MURPHY, J. B., and TAYLOR, H. D.: The lymphocyte in natural and induced resistance to transplanted cancer. III. The effect of x-rays on artificially induced immunity. J. Exper. Med. 28: 1–9, 1918.

(12) DEMPSTER, W., LENNOX, B., and BOAG, J. W.: Prolongation of survival of skin homotransplants in the rabbit by irradiation of the host. Brit. J. Exper. Path. 31: 670–679, 1950.

(13) LORENZ, E., CONGDON, C., and UPHOFF, D.: Modification of acute irradiation injury in mice and guinea-pigs by bone marrow injections. Radiology 58: 863–877, 1952.

(14) PREHN, R. T.: Tumors and hyperplastic nodules in transplanted mammary glands. J. Nat. Cancer Inst. 13: 859–871, 1953.

(15) REINHARD, M. C., MIRAND, E. A., GALTZ, H. L., and HOFFMAN, J. G.: Mouse strain differences in response to radiation. Proc. Soc. Exper. Biol. & Med. 85: 367–370, 1954.

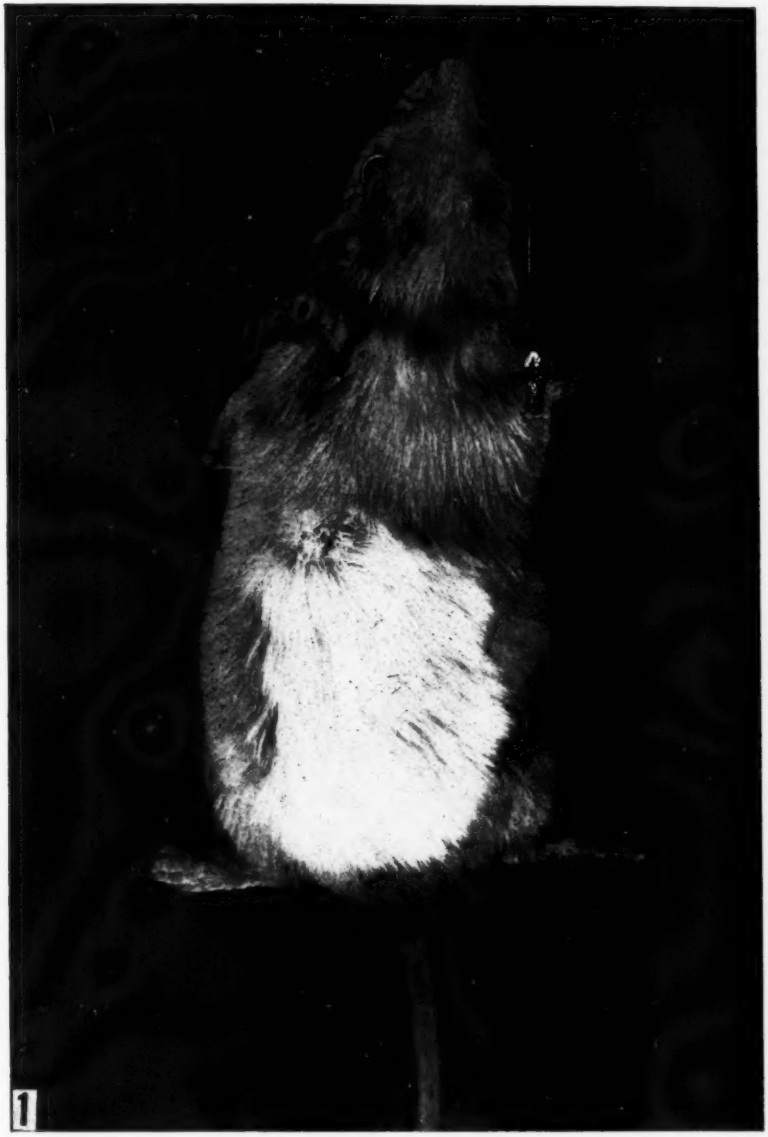
(16) PREHN, R. T., and MAIN, J. M.: A comparison between heterozygous and homozygous skin homografts. J. Nat. Cancer Inst. 15: 191–194, 1954.

(17) BILLINGHAM, R. E., BRENT, L., and MEDAWAR, P. B.: Actively acquired tolerance of foreign cells. Nature 172: 603–606, 1953.

(18) JACOBSON, L. O.: Evidence for a humoral factor (or factors) concerned in recovery from radiation injury. *A review.* Cancer Res.: 12: 315–325, 1952.

PLATE 64

FIGURE 1.—DBA/2JN mouse with BALB/cAnN skin graft after administration of high dosage X radiation (800 r) and (BALB/cAnN × DBA/2JN)F_1 bone marrow. The photograph was taken 150 days after skin grafting.



Main and Prehn 1029

320174—55——20

Effect of Local Roentgen Irradiation on the Formation of New Capillaries After Injury[1]

RUTH M. MERWIN *and* ELIZABETH L. HILL, *National Cancer Institute,[2] Bethesda, Md.*

The decreased capacity of endothelium in irradiated areas to form new capillaries (*1–3*) may play a role in certain postirradiation changes. For instance, it might be a factor in the deleterious effect of irradiation on blood vessels in normal tissues, an important effect because it may limit the dose of radiation that can be given to a tumor (*4*). Or it might be one of the causes of poor healing of surgical incisions in irradiated areas (*5*). More must be known, however, about this decreased capacity of irradiated endothelium to grow before its role can be evaluated.

The object of the experiments reported here was to study the effect of single doses of 400 to 1,500 r on the capacity of endothelium to produce new capillaries. An area of mouse skin was irradiated and within 6 hours was injured either by burning or by placing a stitch of tantalum wire through the skin. Observations were made on the formation of new capillaries in these irradiated injured areas.

Materials and Methods

Materials and Methods Used in the Study of New Capillaries in Burned Areas

Twenty-four male mice of strain BALB/cAnN ranging in age from 3 to 6 months were used.

In order to follow the sequence of changes in living animals a transparent chamber was placed in the skin of each mouse by a method described by Algire and Legallais (*6*). A plastic splint holds the skin away from the body in a double-layered flap along the middorsal line. An incision is made in one of these layers of skin and a mica window is placed under the hole. Through this hole the panniculus carnosus muscle layer of the intact layer of skin is seen.

Roentgen irradiation was administered through the window of the transparent chamber to a circular area of the skin 1 cm. in diameter,

[1] Received for publication November 1, 1954.

[2] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

while the rest of the mouse was shielded. Single doses from 400 to 1,500 roentgens were delivered. The physical factors were as follows: 185 KVP, 170 V, and 20 Ma., 800 r per minute at a focus skin distance of 14.5 cm. through 0.25 mm. Cu and 0.55 mm. Al filters.

Each burn was made through the mica window of a transparent chamber by exposing the tissues for 2 to 6 seconds to light rays which were focused by a microscope condenser and directed through a quartz rod. The flat polished tip of the rod approximately 1 mm. in diameter rested against the mica window.

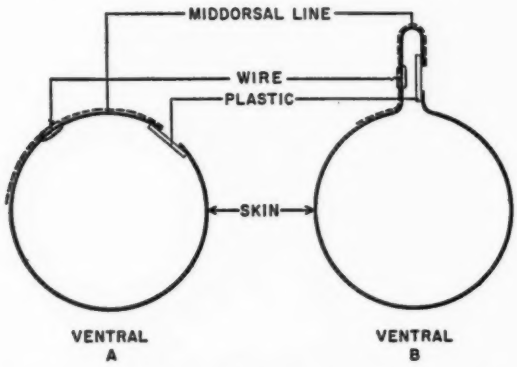
Materials and Methods Used in the Study of New Capillaries Around Stitches of Tantalum Wire

Twenty-seven inbred male mice of strains C3H/HeN and BALB/cAnN were used. They ranged in age from 4 to 12 months but most were 5 to 7 months old.

Several weeks prior to irradiation a window was prepared in the skin by a technique previously described in detail (7). A cut was made in the skin in the middle of the side of each mouse and a circular piece of plastic was inserted through this cut and centered under it.

At the time of irradiation each mouse was placed in a holder that had a slit along the top and the dorsal skin was pulled up from the body through this slit to form a double layer parallel to the middorsal line with the window in one layer and at the edge of the area to be irradiated. During irradiation the double layer of skin was held up by plastic adhesive tape stuck to the hair on the entire surface of both layers. A dose of 500 to 1,500 roentgens was delivered to the double layer of skin while the body of the mouse was shielded. The area of skin irradiated was approximately 2 by 3 cm. The physical factors were: 200 KVP, 240 V, and 15 Ma., 355 r per minute at a focus skin distance of 25 cm. through 0.25 mm. Cu and 0.55 mm. Al filters. Usually four mice of the same strain and age were studied in a group. In each group one mouse received no irradiation and the other 3 received 500 r, 1,000 r and 1,500 r, respectively. After irradiation the skin was allowed to return to its normal position.

Within 6 hours after irradiation a stitch of two strands of tantalum wire was pulled with a fine needle through a small double fold of skin in the irradiated area where the hairs had been clipped. The skin was allowed to return to its normal position so there was no puckering in the tissue and the cut ends of the wires were twisted together. The stitch was located as shown in text-figure 1, *A*, so that when the skin was drawn up in a double fold as is shown in *B* a portion of the stitch 1 to 3 mm. long embedded in the avascular subpannicular layer of connective tissue could be seen through the window. The skin was permitted to remain in its normal position, except when it was drawn up for observations of the area of the stitch through the window. Observations were made almost daily while the changes in the vessels were rapid, but as the changes became slower observations were made less frequently.



TEXT-FIGURE 1.—Diagrammatic cross section of a mouse at the middle of the body to show the relationship of the plastic window and the wire to the mouse's skin. In *A* the skin is in its normal position and in *B* the skin is in a position so that the wire can be seen through the window. The irradiated portion of the skin is indicated by a row of dashes.

Observations and Results

Injury by Local Burns

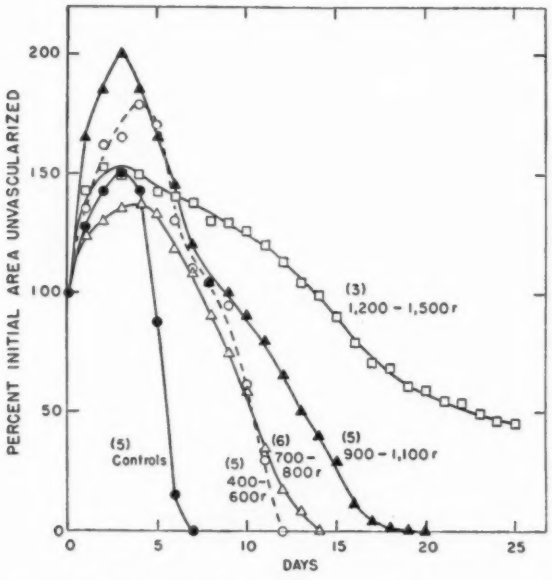
Each burn destroyed most of the vessels in a small area of the subcutaneous-muscle layer, which is the first layer of vascularized tissue under the mica window of the transparent chamber. Most of the new capillaries grew into the burned areas from the surrounding uninjured muscle, but in some burns, some of the capillaries were derived from vessels in other layers of tissue. A few remnants of the original vessels of the muscle layer were often found scattered through the burned areas. Migratory activity of the endothelial cells was indicated by the frequent changes in the pattern of anastomoses between adjacent remnants.

Within the first few hours after burning, no circulation could be seen in an area ranging from 0.14 mm.^{2} to 2.4 mm.^{2}. This unvascularized area was called the initial area. It increased during the next few days and then began to decrease. Measurements were made about every other day until the unvascularized area became smaller than 0.04 mm.^{2} when the burn was considered completely revascularized.

The measurements for each burn were expressed as a percentage of the initial area.[3] These percentages were plotted against time for each animal and a smooth curve was drawn, freehand, to give as good a "fit" as possible to these points. The percentages for days on which no observations had been made were interpolated from these curves. A median curve was obtained for all the smoothed curves for animals that received one dose level of irradiation (*i.e.*, 400–600 r, 700–800 r, 900–1,000 r and

[3] This method has some deficiencies because the unvascularized areas associated with small burns seemed to increase percentagewise more than those associated with larger burns. Increases in the absolute sizes of the unvascularized areas might at times give a clearer concept of the changes taking place.

1,200–1,500 r) by taking the median value for each day (text-fig. 2).[4] The median was used because of the wide range of variation in the data and because of the skewness of the data, which would distort the arithmetic mean.



TEXT-FIGURE 2.—The effect of irradiation dose on area of unvascularized tissue at daily intervals after burning. The values plotted are medians taken from the smoothed curves for individual animals. The number of animals in each experimental group is shown by a number in parentheses. The range of days for each median day for complete revascularization is as follows: (controls) 6–9 range, 7 median; (400–600 r) 10–15 range, 12 median; (700–800 r) 10–22+ range, 14 median; (900–1,000 r) 11–27+ range, 18 median; (1,200–1,500 r) 25–35+ range, 28+ median.

The increase in the areas of nonvascularized tissue in burns varied greatly both in the nonirradiated and in the irradiated animals. No clear relation seemed to exist between this increase and the dose of radiation. The time after burning when the area of nonvascularized tissue started to decrease also could not be related with the dose of radiation received.

The rate of decrease in area of nonvascularized tissue was inversely related to the dose. Thus the dose was directly related to the time required for a complete revascularization of the burned area.

Four [5] of the burns in areas that had received 1,000 r, 1,200 r or 1,500 r were not completely revascularized after 27 to 39 days when observations were discontinued. At this time the tissues in the nonvascularized areas

[4] The authors would like to acknowledge the assistance of Mr. Marvin Schneiderman of the Biometry and Epidemiology Section, National Cancer Institute, in the analysis of these data.

[5] One of these burns that received 1,500 r was not included in text-figure 2 because the initial nonvascularized area was much smaller than that of the other burns.

were translucent and very little tissue seemed to be left in the muscle layer. The nonvascularized area of two of these burns was still slowly decreasing but in the other two, in an animal that had received 1,500 r, the area had stopped decreasing.

The network of new vessels in burns was sparser after doses of 700 or more roentgens than it was after lower doses.

Injury by Stitches of Tantalum Wire

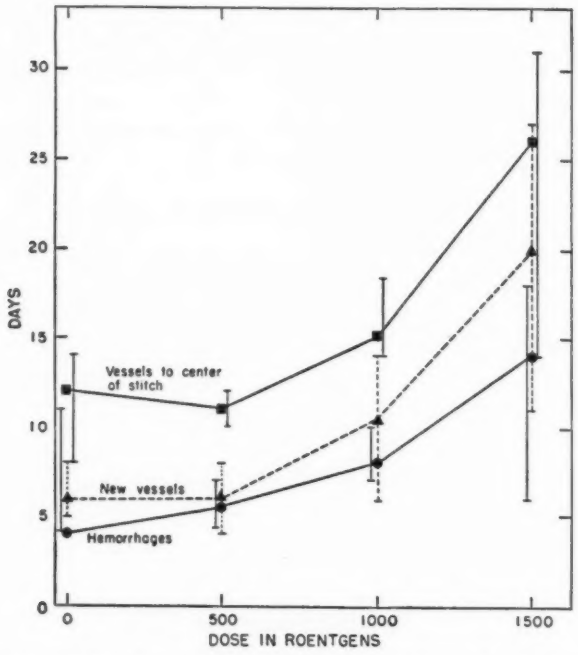
In both irradiated and nonirradiated animals a coating of exudate formed around each wire. The coating ranged in thickness from approximately 0.2 to 1.3 mm. Small hemorrhages appeared around the edge of this coating usually a few days before new capillaries appeared within it. These hemorrhages and new capillaries were seen first at the ends of the stitch where the wire passed through the skin and muscle. While the new capillaries were growing toward the center of the stitch, hemorrhages and more new capillaries usually appeared along the sides. After invasion by capillaries the coating of exudate lost its distinct edges and was replaced by granulation tissue.

A dose of 500 r may have slowed the first appearance of hemorrhages slightly but it had no effect on the time of appearance of new vessels nor on their progress through the coating (text-fig. 3). With increases in dose over 500 r there was an increasing delay in vascularization. After 1,000 or 1,500 r the new network of vessels was sparser than it was after lower doses.

Discussion

As the dose of radiation was increased from 500 r to 1,500 r the rate of revascularization of burned areas decreased, and the interval before vessels appeared in the coating of exudate around tantalum wires increased. The factors that caused the large range in the responses were not clear. Neither was it clear why a dose of 500 r delayed the revascularization of burned areas but seemed to have little effect on the vascularization of the coating of exudate around wires.

Irradiation may have affected the growth of new capillaries indirectly by altering the tissue into which the capillaries grew. This is suggested by the finding that after 1,200 to 1,500 r the tissues in burned areas were changed by 3 weeks after burning. They were translucent and there seemed to be very little tissue left in the muscle layer. The vessels growing into these visibly changed tissues grew slowly or stopped growing. The possibility should be considered that as the dose of radiation increased, the tissue in the burned areas became altered so that the rate of growth of new vessels into it was slower. The behavior of vessels in the coating of exudate around wires in areas that received 1,500 r was different from that in burns. Although the capillaries in burns grew slowly, or stopped growing, the capillaries in the exudate coating around wires grew to the center of the stitch apparently at the same rate as at lower doses. This may be related to the finding that the coating, unlike the burned tissues,



TEXT-FIGURE 3.—Median day and range of days at each dose of radiation for (1) the first appearance of hemorrhages; (2) the first appearance of new capillaries; and (3) the first penetration of new capillaries to the center of the stitch. Each median for the nonirradiated animals is based on 12 observations. All other medians are based on 4 to 7 observations.

showed no visible change until it became vascularized. It is possible that the effect of irradiation on capillaries around wires was mainly a direct one.

One cannot say whether the sparseness of the new network of vessels after higher doses is a direct or an indirect effect of irradiation.

Pohle (*8*) reports that wounds made immediately before or after a dose of 1,000 r healed slowly. Dobbs (*9*) found that after 1,000 r the tensile strength of the scars decreased and Lawrence *et al.* (*10*) report a delay in the attainment of maximum tensile strength after 2,000 r. The effect of irradiation on the formation of new capillaries may have contributed to these changes in healing.

Summary and Conclusions

Studies were made on the effect of irradiation on the formation of new capillaries in injuries in the skin of mice. Doses of 400 to 1,500 roentgens were given, and within 6 hours the skin was injured by a small burn or by inserting a stitch of tantalum wire.

A dose of 500 r slowed the rate of revascularization of the burned areas but had no effect on the time of vascularization of the coating of exudate that formed around tantalum wires.

As the dose of radiation was increased from 500 to 1,500 roentgens the rate of revascularization of burned areas decreased and the interval before capillaries began to invade the coating around wires increased.

The new network of vessels in both types of injuries was sparser following doses of radiation that exceeded 700 roentgens than it was following lower doses.

References

(*1*) Borak, J.: Radiation effects on blood vessels. Radiology 38: 481–492, 607–617, 718–727, 1942.

(*2*) Takahashi, T.: The action of radium upon the formation of blood capillaries and connective tissue. Brit. J. Radiol. 3: 439–445, 1930.

(*3*) Merwin, R. M., Algire, G. H., and Kaplan, H. S.: Transparent-chamber observations of the response of a transplantable mouse mammary tumor to local roentgen irradiation. J. Nat. Cancer Inst. 11: 593–623, 1950.

(*4*) Paterson, R.: The Treatment of Malignant Disease by Radium and X Rays. London, Edward Arnold & Co., 1948.

(*5*) Dobbs, W. G. H.: A statistical study of late effects of heavy roentgen irradiation on healing of skin wounds. Am. J. Roentgenol. 45: 107–108, 1941.

(*6*) Algire, G. H., and Legallais, F. Y.: Recent developments in the transparent-chamber technique as adapted to the mouse. J. Nat. Cancer Inst. 10: 225–253, 1949.

(*7*) Merwin, R. M., and Hill, E. L.: Fate of vascularized and nonvascularized subcutaneous homografts in mice. J. Nat. Cancer Inst. 14: 819–839, 1954.

(*8*) Pohle, E. A., Ritchie, G., and Wright, C. S.: Studies of the effect of roentgen rays on the healing of wounds. Behavior of skin wounds in rats under pre- or post-operative irradiation. Radiology 16: 445–460, 1931.

(*9*) Dobbs, W. G. H.: Statistical study of the effect of roentgen rays on wound healing. Am. J. Roentgenol. 41: 625–632, 1939.

(*10*) Lawrence, W., Jr., Nickson, J. J., Warshaw, L. M.: Roentgen rays and wound healing; an experimental study. Surgery 33: 376–384, 1953.

Study of Recovery at Low Temperature of X-Irradiated *E. coli* Cells [1]

A. W. Pratt, W. S. Moos,[2] *and* M. Eden,[3]
National Cancer Institute,[4] *Bethesda, Md.*

There is general agreement that the effects of ionizing radiation on biological systems may be modified by changing the physical or chemical environment of the organism. The present work was undertaken to determine the comparative recovery patterns obtained when distilled-water suspensions, citrate suspensions and broth suspensions of *Escherichia coli* cells were exposed to X irradiation and were subsequently maintained at low temperatures.

In this study it was considered that recovery could be determined by measuring the change that occurs in the number of viable cells [5] which, as a function of time stored in the cold, recover the ability to form macrocolonies. It was also considered that storage at low temperatures would prevent cell multiplication from obscuring the recovery pattern. Barber (*4*) has shown that mitosis of *E. coli* cells is reduced with decreasing temperature and is essentially zero at 4° C. A review of the literature showed that there is no general agreement as to what effect storage of the irradiated cells at low temperature may have toward modifying the recovery pattern (*5–10*).

Experimental Procedures

In a typical experiment, cultures were prepared by inoculating horsemeat infusion broth with *E. coli* (Strain B)[6] and incubating for 16 hours at 36° C. The cultures were then removed from the incubator, centrifuged and repeatedly washed with distilled water, and recentrifuged to remove as completely as possible all adsorbed material. The broth-free water suspension of bacteria was shaken in the presence of small glass

[1] Received for publication November 1, 1954.

[2] *Present address:* Department of Radiology, University of Illinois, Urbana, Ill.

[3] *Present address:* Postdoctorate Fellow, Department of Mathematics, Princeton University, Princeton, N. J.

[4] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

[5] In these studies a viable cell is taken to mean the ability to multiply and form a macrocolony. Relative survival is measured by counting the number of colonies produced by the test object and comparing this number with the number of colonies produced by a control.

It is recognized that the recovered cells in this study would also include those cells that may have undergone a "lethal" mutation which would not express itself until the original cell had undergone several divisions. The problem of lethal mutations has been discussed in detail by several workers (*1–3*).

[6] Obtained from Dr. L. Labaw, National Institutes of Health.

beads to insure a homogeneous suspension of bacteria and then transferred in aliquots of 0.1 ml. into each of six small test tubes. To one pair of these tubes, 0.1 ml. of sterile broth was added to each tube; to the second pair, 0.1 ml. of 0.05 *M* Na_3 citrate (*p*H 7.3) was added to each tube; and to the third pair, 0.1 ml. of distilled water was added to each tube. One tube from each of the three sets was selected as the experimental tube for exposure to X irradiation; the three remaining tubes, one from each set, served as controls.

After the desired X-irradiation dose had been delivered to the experimental samples, they were diluted $1:10^7$ times with distilled water. The control samples were diluted in an identical manner. From each of these diluted suspensions, plate cultures were prepared (0.1 ml. per petri dish) and incubated for 24 hours at 36° C. The number of colonies was determined by the photographic method of Moos (*11*). The remaining bacterial suspensions were stored in a refrigerator at 4° to 6° C. and used for subsequent platings at regular 24-hour intervals for an over-all period of 6 days.

A second set of experiments was conducted which differed from the experiments described above only in that sterile broth instead of distilled water was used as the diluent and storing medium.

The radiation was delivered by 186 KVP X-ray unit at a dose rate of 250 r per minute. For filtration of the beam, 0.25 mm. copper and 0.55 mm. aluminum were employed. A total dose of 3,000 r, measured in air, was given all three experimental samples.

Results

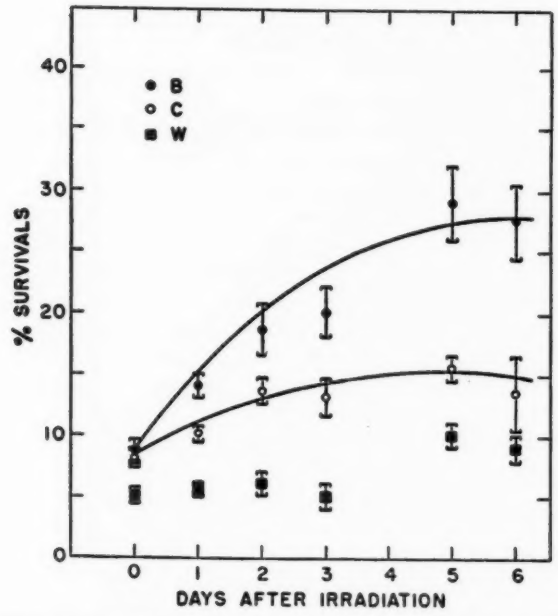
Study of the control cultures stored at 4° to 6° C. showed that proliferation of the cells did not occur; on the contrary, there was a steady decline in the number of viable cells found in the successive daily examinations of the suspensions. The rate of dying was different for each of the bacterial suspensions—being most marked in citrate and least pronounced in the broth suspensions. The normal death rate appeared to be proportional to the number of viable cells present and can be described by the general form:

$$-\frac{dx}{dt}=k_1x$$

where $-\frac{dx}{dt}$ is the rate at which the number of viable cells decreased with time and x is equal to the number of viable cells present.

The normal death-rate constant, *i.e.*, k_1, when computed by the method of least squares, is found to be equal to 0.05, 0.05 and 0.12 for broth suspensions, distilled-water suspensions and citrate suspensions, respectively.

The observations made on the irradiated cultures are shown graphically in text-figures 1 and 2. Each point plotted on the curves represents the mean of all the assays, and, in addition, the standard error of the mean value is indicated.



TEXT-FIGURE 1.—●Cells irradiated in broth—diluted with water.
○Cells irradiated in citrate—diluted with water.
■Cells irradiated in water—diluted with water.

Text-figure 1 shows the immediate survivor ratio and the subsequent recovery ratio for the X-irradiated *E. coli* cells stored at 4° to 6° C. as a function of time when the suspensions have distilled water as the diluent and suspending medium. It is apparent that the number of survivor cells present immediately after X-irradiation exposure was approximately equal in the broth and citrate systems. The number of viable cells in the distilled-water suspension found immediately after X irradiation was somewhat less than the others. Successive daily examinations of the irradiated suspensions revealed that there were a significant number of cells in both the broth suspensions and the citrate suspensions which, with time, recovered their ability to divide when stored at 4° to 6° C. These data show that if the X-irradiated suspensions are stored at low temperature with no change in their chemical environment save for dilution with distilled water, there is extensive recovery of cell viability in the nutrient broth suspension, a lesser recovery of viability of the irradiated cells in the citrate suspensions and questionable recovery in the distilled-water suspensions. It has been observed, however, that when the control cultures are stored at this low temperature the number of viable cells becomes progressively less; thus the interpretation of these observations requires a consideration of the "normal death rate" which characterizes the storage of viable cells at these low temperatures in the respective suspending media. If it can be assumed that the viable cells in the ir-

radiated suspensions will die at a rate comparable to that observed for the viable cells in the control suspensions, it follows that these data represent the net recovery pattern. The recovery pattern should be equal to the number of viable cells found at any examination plus the number of irradiated cells which have recovered and may have subsequently died. Such an interpretation demands that the recovery pattern for the X-irradiated broth suspensions stored at 4° to 6° C. with distilled water serving as the diluting and storing medium, should be somewhat greater than that recorded in text-figure 1. The normal death-rate constant for citrate is twice as large as that for nutrient broth, and even after dilution with distilled water it is doubtful that the toxic influence of citrate would be greatly altered. Therefore, it would seem that the recovery pattern for the irradiated citrate suspension should be more nearly comparable to the recovery pattern of the irradiated broth suspensions if the toxic effect of citrate could be reduced. The observation that the number of viable cells in the irradiated distilled-water suspensions apparently remains essentially constant during the experimental period, could result only if there were sufficient cells able to recover to replace those being lost at the normal rate of death.

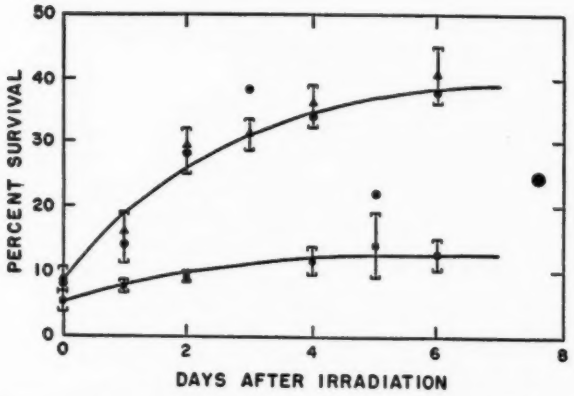
A second series of experiments were undertaken to ascertain whether or not recovery in all the irradiated suspensions could be experimentally shown. The experimental procedure was identical to the first series of experiments, except that nutrient broth was used for the diluent and storage medium. It was thought that dilution of the citrate with broth would substantially reduce the toxic effects of the citrate ion.

The observations made in the second series of experiments are recorded in text-figure 2. It can be seen that the number of immediate survivors in the X-irradiated nutrient broth, citrate and distilled-water suspensions was essentially the same as that seen in text-figure 1. This result is expected since they represent similar experiments and again show that more protection was afforded the irradiated cells by the presence of nutrient broth and dissolved citrate ion in comparison to distilled-water suspensions.

It was found that the recovery patterns were significantly altered for all of the irradiated suspensions. The net recovery patterns for both the irradiated broth suspension and the citrate suspension were experimentally indistinguishable and the degree of recovery was greater than that found when the irradiated suspensions were diluted with and stored in distilled water.

The irradiated distilled-water suspensions show a significant degree of recovery. However, it is considerably less than that seen in the irradiated broth and citrate suspensions.

The presence of dissolved solute such as citrate and broth particulates in the bacterial suspensions will effectively compete with the bacterial cells during the irradiation exposure. The data show the extent of this protection in that the number of immediate survivors in the "unprotected" distilled-water suspension are less than the number of immediate survivors



TEXT-FIGURE 2.—▲Cells irradiated in broth—diluted with broth.
●Cells irradiated in citrate—diluted with broth.
■Cells irradiated in water—diluted with broth.

in the "protected" suspensions containing broth or citrate. The effect of the protecting solutes is clearly demonstrated by comparing qualitatively the difference in percent survival between the protected and unprotected suspensions after the 6-day recovery period.

Discussion

The results of these studies indicate that a progressive recovery in the number of viable *E. coli* cells, capable of macrocolony formation, occurs when the irradiated suspensions are stored at 4° to 6° C. for 6 days. Some degree of progressive recovery of viability was found in all test suspensions examined.

Stapleton, Billen and Hollaender (*7*) have reported extensive studies on the response of certain strains of irradiated *E. coli* cells to storage in the cold. They find a significant recovery when the bacterial cells are stored at temperatures below optimal growth temperature. These authors report no significant recovery at temperatures of 6° C., or lower. Our data indicate appreciable recovery in the temperature range of 4° to 6° C. It is possible that Stapleton *et al.* would have observed some recovery at the lower temperature had their cultures been stored for periods longer than 24 hours.

From our data we conclude that the "lethal" manifestation of irradiation on bacterial cells can be arrested if the cells are metabolically depressed by storage in the cold. In addition, it is necessary to conclude that a considerable number of cells will be able, during the cold storage, to repair the irradiation damage and recover viability. The observations of Stapleton *et al.*, of Cook (*9*) and of Latarjet (*12*) seem to be in general agreement with these conclusions. The experiments of Schrek (*13*) with thymus cells and Strangeways and Fell (*14*) with embryonic fowl explants also lend support to our interpretations.

Stapleton *et al.* have observed that irradiated cells do not recover in the cold in an organic salts-glucose medium that will sustain growth and cell division in control cells. They suggest the existence of a special nutritive requirement essential to the recovery process. Our results reveal that optimal recovery is obtained when the irradiated suspensions are stored in broth. However, a significant recovery is seen in the dilute citrate suspension and some recovery is seen in the distilled-water suspensions. The dependence of recovery on the chemical environment is not clear. The marked recovery found in the citrate suspensions is difficult to reconcile with the suggestion of a special nutritive requirement essential to recovery, unless it can be assumed that there had been storage previous to irradiation of the nutritive requirement or precursors of the substance from which the nutritive requirement could be synthesized by the exposed cells. Our observations tend to favor the hypothesis that the recovery mechanism is intrinsic in the cell and that the presence of a complete medium sustains the recovery process at an optimal rate.

Our data appear to have the general form of a first-order process in which the rate of appearance of viable cells is directly proportional to the cells capable of recovery. The data obtained by Latarjet on yeast cells stored in the cold may be similarly interpreted. It may be noted that the photoreactivation of cells after ultraviolet irradiation is also a first-order process depending on the number of "photo reactivable" organisms (*15*). The rate dependence of a biological process upon the number of organisms capable of participating in the process in question is a common observation.

Determination of the recovery patterns of the individual suspensions clearly indicates that the protective effect of the dissolved organic substances is considerably greater than that suggested by the number of immediate survivors or even the number of 24-hour survivors in irradiated cultures maintained under these experimental conditions. The recovery process and, thus the total protection, may be quantitated within the limits of certain assumptions.

From the experimental observations it would appear that following irradiation the bacterial cells in the individual suspensions can be divided into four groups as follows: *Group 1.*—Consists of viable bacterial cells capable of cell division immediately after X-irradiation exposure. *Group 2.*—Consists of bacterial cells which are "arrested" in that they are incapable of cell division immediately after X irradiation but which are capable of recovering this vital function with storage in the cold. *Group 3.*—Consists of bacterial cells which were immediately killed by X irradiation. *Group 4.*—Consists of bacterial cells that die during the period of storage in the cold.

It has already been assumed that the normal death rate can be described by a first order kinetic expression (see page 1040). In addition, it has been assumed that the recovery rate of the arrested cells under these experimental conditions is a first-order process dependent on the number of arrested cells present immediately after X irradiation which are capable of recovering the vital function of cell division.

The change in the number of viable cells present at any time, t, may be expressed as the net effect of these two processes

$$\frac{dx}{dt} = k_2 y - k_1 x \qquad [1]$$

and the change in the number of cells capable of recovering the function of cell division is

$$\frac{dy}{dt} = -k_2 y - k_3 y \qquad [2]$$

$$= -k_4 y$$

where

x=The number of cells capable of cell division at any time $t \geq t_0$.
x_0=The number of cells capable of cell division immediately after X irradiation when the recovery time $t = t_0 = 0$.
y=The number of arrested cells capable of recovery of the cell division function at any time $t \geq t_0$.
y_0=The number of cells capable of recovery of the cell division function immediately after X irradiation when the recovery time $t = t_0 = 0$.
k_1=The rate constant for death of normal cells,
k_2=the rate constant for recovery of the arrested cells,
k_3=the rate constant for death of cells capable of recovery
and $k_4 = k_2 + k_3$.

The boundary condition assumed was that the number of living cells capable of cell division, x, decreases to zero as time of storage in the cold increases indefinitely.

Integrating equation [2] and substituting for y in equation [1] will give a final expression for x:

$$X = \begin{cases} c_1 e^{-k_1 t} + \dfrac{k_2 y_0}{k_1 - k_4} e^{-k_4 t} + c_2 & \text{when } k_1 \neq k_4 \\ c_1 e^{-k_1 t} + k_2 y_o{}^t e^{-k_1 t} + c_2 & \text{when } k_1 = k_4 \end{cases} \qquad [3]$$

where c_2 is equal to zero by the boundary condition.

It is experimentally impossible to determine the death rate of the unrecovered cells. Therefore it has been necessary to make an arbitrary assumption with regard to k_3—leaving the three adjustable parameters y_0, k_2 and c_1—as follows:

Assumption (*a*) that the cells capable of recovery do not die before recovery is effected or $k_3 = 0$, hence $k_4 = k_2$.

Assumption (*b*) that the unrecovered cells capable of recovery die at the same rate as that at which the viable cells die,
or

$$k_4 = k_1 + k_2.$$

Equation [3] may be employed to calculate the proportion of arrested cells present immediately after irradiation. The computation for the results illustrated in text-figures 1 and 2 are presented in table 1. Since the numerical process used to fit the equation to the data permits considerable uncertainty in the values of the parameter, these percentages can only be considered to be approximate.

The apparent fit of the data to these theoretical equations as shown by the curves drawn in text-figures 1 and 2 cannot be adduced as proof of the validity of the hypotheses and the statements that may be derived from these hypotheses. The analysis indicates, however, that arrested cells can die without recovering.

TABLE 1

Irradiated in	Diluted	Percent nonviable	Percent arrested	Percent viable
Assumption (a)				
Broth	H_2O	59.8	31.5	8.7
Citrate	"	72.7	19.1	8.2
H_2O	"	— — —	— — —	5.0
Broth	Broth	44.0	47.5	8.5
Citrate	"	44.0	47.5	8.5
H_2O	"	81.4	13.6	5.0
Assumption (b)				
Broth	H_2O	49.8	41.5	8.7
Citrate	"	55.0	36.8	8.2
H_2O	"	— — —	— — —	5.0
Broth	Broth	30.0	61.5	8.5
Citrate	"	30.0	61.5	8.5
H_2O	"	78.0	17.0	5.0

Summary

The recovery of *Escherichia coli* cells from X irradiation has been examined as a function of time stored in the cold in distilled water suspensions, citrate suspensions, and broth suspensions. Some degree of recovery was observed in all irradiated cell suspensions. It is suggested that the mechanism of recovery is intrinsic to the cell.

References

(*1*) DEMEREC, M., and CAHN, E.: Studies on mutability in nutritionally deficient strains of *Escherichia coli*. I. Genetic analysis of five auxotrophic strains. J. Bact. 65: 27–36, 1953.

(*2*) NEWCOMBE, H. B.: The delayed appearance of radiation-induced genetic change in bacteria. Genetics 38: 134–151, 1953.

(*3*) GLUECKSOHN-WAELSCH, S.: Lethal factors in development. Quart. Rev. Biol. 28: 115–135, 1953.

(*4*) BARBER, M. A.: The rate of multiplication of bacillus coli at different temperatures. J. Infect. Dis. 5: 379–400, 1908.

(5) HENSHAW, P. S.: Further studies on the action of roentgen rays on the gametes of Arbacia punctulata. VI. Production of multipolar cleavage in the eggs by exposure of the gametes to roentgen rays. Am. J. Roentgenol. 43: 923–933, 1940.

(6) LAMARQUE, P., and GROS, C.: The eggs of the Bombyx-mori as material for radiobiological research. Brit. J. Radiol. 18: 293–296, 1945.

(7) STAPLETON, G. E., BILLEN, D., and HOLLAENDER, A.: Recovery of x-irradiated bacteria at suboptimal incubation temperatures. J. Cell. & Comp. Physiol. 41: 345–357, 1953.

(8) ALLEN, B. M., SCHJEIDE, O. A., and HOCHWALD, L. B.: The influence of temperature upon the destruction of hematopoietic cells of tadpoles by x-irradiation. J. Cell. & Comp. Physiol. 38: 69–82, 1951.

(9) COOK, V. E.: Influence of low temperature on recovery from roentgen rays. Radiology 32: 389–393, 1939.

(10) KELNER, A.: Effect of visible light on the recovery of streptomyces griseus conidia from ultraviolet irradiation injury. Proc. Nat. Acad. Sc. 35: 73–79, 1949.

(11) MOOS, W. S.: Variation of irradiation effects on microorganisms in relation to physical changes of their environment. J. Bact. 63: 688–690, 1952.

(12) LATARJET, R.: Action du froid sur la reparation des radio-lesions chez une levure et chez une bacterie. Compt. rend Acad. Sc. 217: 186–188, 1943.

(13) SCHREK, R.: Studies in vitro on the physiology of cells. Factor affecting the delayed cytocidal action of x-rays. J. Cell. & Comp. Physiol. 28: 277–304, 1946.

(14) STRANGEWAYS, T. S. P., and FELL, H. B.: Study of direct and indirect action of X-rays upon tissues of embryonic fowl. Proc. Roy. Soc., s.B 102: 9–29, 1927.

(15) DULBECCO, R.: Experiments on photoreactivation of bacteriophages inactivated with ultraviolet radiation. J. Bact. 59: 329–347, 1950.

Long-Term Effects of Acute and Chronic Irradiation in Mice. I. Survival and Tumor Incidence Following Chronic Irradiation of 0.11 r Per Day[1]

EGON LORENZ,[2] JOANNE WEIKEL HOLLCROFT, ELIZA MILLER, CHARLES C. CONGDON, *and* ROBERT SCHWEISTHAL,[3,4] *National Cancer Institute,[5] Bethesda, Md., and The Division of Biological and Medical Research, Argonne National Laboratory, Chicago, Ill.*

This is the first of a series of papers attempting to clarify points raised in earlier work on the ultimate effects of chronic and acute irradiation. The complete results of these early experiments on chronic γ irradiation have been published in the National Nuclear Energy Series (*1–6*). In one of these studies, when 45 LAF_1 mice were exposed to chronic γ irradiation at a rate of 0.11 r per 8-hour day for duration of life, an increase in life span over that of the 59 controls was seen. This increase was not statistically significant, however.

From a theoretical standpoint a shortened life span may be predicted even at this low dose (*7*). All radiation produces deleterious effects, some of which are reparable and some of which are permanent and cumulative. Even if the concept of a threshold dose, below which the damage is negligible, is accepted, a lengthened life span cannot be predicted. Therefore, a lengthened life span may alter some concepts of radiation effects. In addition, the earlier work indicated that this low dose was carcinogenic. Hence, the repetition of the 0.11 r exposure using a larger number of animals was undertaken.

Experimental Methods

$(C_{57}L \times A)F_1$ mice, hereafter referred to as LAF_1, were used in this study. At one month of age the experimental animals were placed in wooden cages in the exposure room where they were exposed for duration of life to 0.11 r per 8-hour day γ radiation from a 100 mg. source of radium. A complete description of the exposure conditions has been given in the report of the original experiment (*2*). The control mice were housed in plastic cages in a room adjacent to the exposure room. Unfortunately, the original control animals developed dermatitis and had to be replaced by other controls almost a year after the start of the experiment. These mice

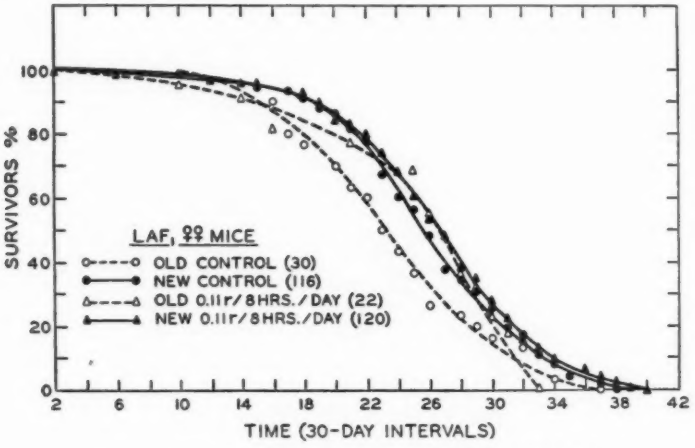
[1] Received for publication October 22, 1954.
[2] Deceased February 12, 1954.
[3] *Present address:* Argonne National Laboratory.
[4] The authors gratefully acknowledge the technical assistance of Bruce L. Weed.
[5] National Institutes of Health, Public Health Service, U.S. Department of Health, Education, and Welfare.

were not directly related to the irradiated mice but were derived from crosses of the same inbred strains.

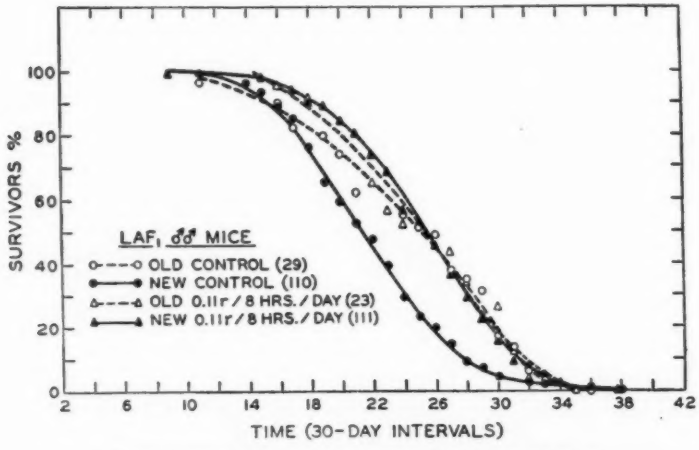
All mice were fed Purina laboratory chow and given tap water *ad libitum*. The mice were weighed weekly. Some were killed and autopsied when they became moribund; other animals found dead were autopsied whenever autolysis was not great. The tissues were fixed in formalin-Zenker's fluid and the sections prepared from them were stained with hematoxylin and eosin.

Results

The findings on the mean survival time, presented in table 1 and text-figures 1 and 2, show that the irradiated males live longer on the average



TEXT-FIGURE 1.—Survival curves of LAF_1 female mice.



TEXT-FIGURE 2.—Survival curves of LAF_1 male mice.

TABLE 1.—*Mean survival time of LAF_1 mice*

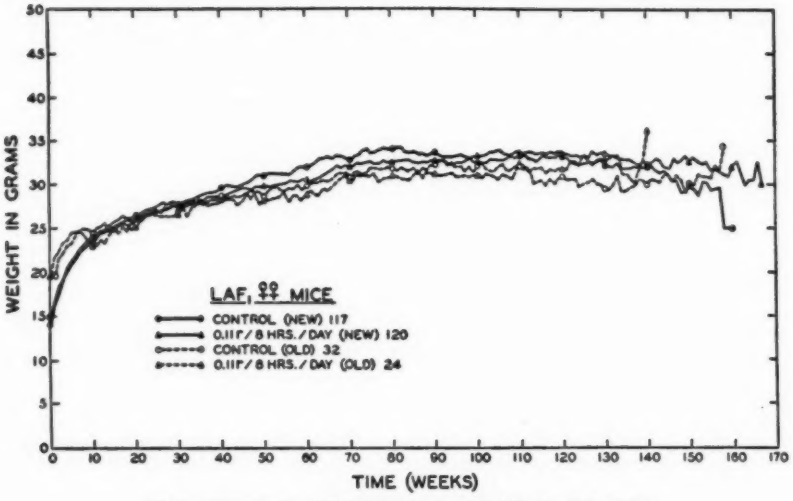
Experiment	Controls			Irradiated			Difference between irradiated and control groups	*P* Irradiated *vs* controls
	No. of mice	Sex	Mean survival time	No. of mice	Sex	Mean survival time		
			days *S. E.**			*days* *S. E.**	*days*	
New	110	♂	683. 5 ± 14. 3	111	♂	783. 1 ± 14. 0	99. 6	0. 01
	116	♀	802. 9 ± 16. 1	120	♀	820. 3 ± 17. 6	17. 4	N. S.†
Old	29	♂	782. 3 ± 34. 2	23	♂	816. 6 ± 31. 2	34. 3	N. S.
	30	♀	763. 9 ± 32. 2	22	♀	809. 7 ± 42. 1	45. 8	N. S.
	59	♂ + ♀	772. 9 ± 23. 3	45	♂ + ♀	813. 2 ± 25. 8	40. 3	N. S.

*Standard error.
†Not significant.

than the control males but that no difference in life span exists between the two groups of females. In the course of the experiment, 2 male and 4 female controls and 1 irradiated male were lost in handling and are not included in the data. Also shown, is the fact that the female controls lived significantly ($P<.01$) longer than the male controls. However, the sex difference seen in the irradiated animals is not significant.

The data from the original (old) experiment are reported here for comparison. No significant difference exists between the mean survival times of the old and the new experiments except in the control male groups.

The weight of the irradiated female mice did not differ greatly from that of the control females (text-fig. 3). However, the irradiated males

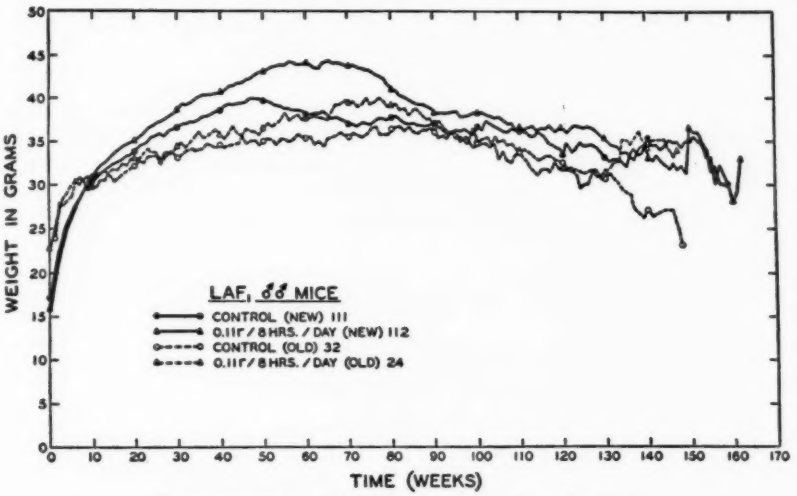


TEXT-FIGURE 3.—Weight curves of LAF_1 female mice.

were heavier than the control males from about 20 weeks to 90 weeks after they were put into the radiation field (text-fig. 4). This weight difference is greater in the present experiment than that found previously,

TABLE 2.—*Percent incidence and type*

Experimental group	Sex	Mice autopsied	Mice with tumors of reticular tissues		Mice with lung tumors		Mice with sarcomas	
			Percent	Mean age at death	Percent	Mean age at death	Percent	Mean age at death
		number		*months*		*months*		*months*
Control	♀	101	43	28	45	29	22	28
Irradiated with 0.11 r	♀	98	67	29	38	30	22	30
Control	♂	83	12	23	37	25	1	27
Irradiated with 0.11 r	♂	94	30	27	52	26	4	23



TEXT-FIGURE 4.—Weight curves of LAF_1 male mice.

and on the whole the males and females are heavier in this study than they were in the old one.

Table 2 shows the incidence of the more frequently occurring tumors and the average age at death of the animals bearing each type of tumor. The incidence of tumors of the reticular tissues was significantly ($P<.01$) greater in the irradiated than in the control mice. Lymphocytic neoplasms made up the bulk of these tumors. There were 27 in the irradiated males; 66 in the irradiated females; and 9 and 35 in the control male and female groups, respectively. The other tumors of the reticular tissues included: 2 myeloid leukemias and 1 reticulum-cell sarcoma in the irradiated males; 1 granulocytic and 1 reticulum-cell sarcoma in the male control group; 1 plasma-cell tumor in the irradiated female group, and 7 granulocytic leukemias; and 1 reticulum-cell sarcoma in the female control group.

The increase in the incidence of the mammary tumors in the irradiated female mice over the controls is also highly significant ($P<.01$). The

of tumors discovered at autopsy

Mice with hepatomas		Mice with mammary carcinomas		Mice with ovarian or testicular tumors		Mice with carcinomas other than mammary		Mice with mast-cell tumors	
Per-cent	Mean age at death	Per-cent	Mean age at death	Per-cent	Mean age at death	Per-cent	Mean age at death	Per-cent	Mean age at death
	months		*months*		*months*		*months*		*months*
10	31	0	.....	4	26	6	30	2	19
5	30	8	27	11	33	2	30	2	35
20	24	0	.....	4	29	3	29	0	.....
15	26	1	28	1	27	2	28	2	22

mammary tumors included 6 adenocarcinomas and 2 adenoacanthomas. In addition 1 adenocarcinoma of the mammary gland appeared in an irradiated male mouse.

The increase in ovarian tumors in the irradiated mice is significant at about the 5-percent level. Half of these tumors were mixed granulosa-cell and tubular-adenoma tumors; in addition there occurred 3 granulosa-cell tumors, 1 tubular adenoma, and 1 cystadenoma. Six of the ovarian tumors occurred as the only tumor in the animal (excluding lung tumors); 3 were accompanied by sarcomas and 1 by a mammary carcinoma.

In addition, the irradiated males showed an increase in lung tumors at about the 5-percent level. The average number of nodules per animal was not calculated.

The susceptibility to infection apparently did not increase following irradiation with 0.11 r. Pneumonia was found in only 2 or 3 mice, but kidney infection and heart disease were found frequently (table 3). Included in the category of kidney infection are pyelonephritis, and papillonecrosis. These were usually associated with amyloid deposits not only in the kidney but also in the liver and spleen. In a very few cases amyloidosis was found without kidney infection. The heart was affected by valvular thrombosis and auricular thrombosis.

TABLE 3.—*Occurrence of kidney and heart lesions*

Experimental group	Sex	Mice with kidney infections		Mice with valvular and auricular thromboses in heart	
		Percent	Mean age at death	Percent	Mean age at death
			months		*months*
Control	♀	29	26	6	26
Irradiated with 0.11 r	♀	35	28	2	35
Control	♂	86	23	12	23
Irradiated with 0.11 r	♂	79	26	15	26

The sex difference in tumor incidence is noteworthy, as is the difference in heart and kidney diseases (table 4). One of the most obvious differences between the males and females is in the occurrence of sarcomas. Whether or not the sarcoma involved the mammary gland was not tabulated because it was felt many more sections of each tumor would be needed to determine this fact. It is probable that most of the sarcomas in the females were of mammary origin. Only 3 of the sarcomas in the irradiated females were not situated subcutaneously; one occurred in the uterus, one in the sternum, and one in the hind leg.

In addition to the tumors appearing in table 2, some miscellaneous tumors appeared. In the female control group a myxoma of the mammary gland was found at 32 months; a hemangioma of the uterus at 33 months; and a leiomyoma of the cervix at 37 months. In the control male group 1 hemangioendothelioma of the liver occurred at 30 months and in the irradiated males a fibroepithelial papilloma of the skin occurred at 30 months.

TABLE 4.—*Sex difference in occurrence of tumors and lesions of the heart and kidney*

Lesion	Controls	Irradiated
Hepatoma	♂ > ♀ ($P<.05$)	♂ > ♀ ($P<.02$)
Lymphoma	♀ > ♂ ($P<.01$)	♀ > ♂ ($P<.01$)
Lung tumor	N. S.*	♂ > ♀ ($P<.02$)
Sarcoma	♀ > ♂ ($P<.01$)	♀ > ♂ ($P<.01$)
Kidney lesion	♂ > ♀ ($P<.01$)	♂ > ♀ ($P<.01$)
Heart lesion	N. S.	♂ > ♀ ($P<.01$)

*Not significant.

Discussion

The data from the males and females were considered separately in this experiment because of the marked sex difference in the mean survival time of the control group. This tendency of the female toward greater longevity has been noted in other strains of mice (*8*, *9*). Since, in the irradiated animals, the life span of the males and the females was essentially the same, it can be argued that chronic irradiation produced a beneficial effect in the male mice. In respect to investigation of the sex difference in life span, both groups of mice were excellently controlled and the results are statistically significant.

On the other hand, the experiment was conducted under certain difficulties due to the fact that it was necessary to procure a new control group about one year after the beginning of the irradiation. This second control group may have been subject to different environmental conditions and therefore the observed difference in life span cannot be accepted without reservation.

Although no single cause of death predominated, pyelonephritis appeared to be a major contributing factor. This typical renal disease, found frequently in some strains of mice, has been described in detail by Dunn (*10*). It has been frequently associated with dermatitis and the incidence has been modified by changing the diet (*11*). Therefore, the incidence in any inbred strain can be expected to vary with changing conditions in the animal room.

The sex difference in renal disease noted in this study may be related to the fact that the kidney of the male mouse differs from the kidney of the female (*12*). However, when Heston and Deringer (*13*) found an incidence of amyloidosis of about 27 percent in LAF_1 mice they observed no sex difference. It was suggested, prior to this experiment, that nephritis and related kidney disease might be less prevalent in the irradiated animals. However, the difference between the irradiated and nonirradiated animals was not statistically significant, either in the case of the males with an extremely high rate of renal disease or in the case of the females with a lower incidence.

From previous findings, it was thought that the mammary tumors, both carcinomas and sarcomas, were associated with granulosa-cell tumors of the ovaries (*3*, *5*). In the present study this association did not hold. More subcutaneous sarcomas occurred in the absence of ovarian tumors than in

their presence. Only 1 mammary carcinoma occurred in the presence of a granulosa-cell tumor while 7 appeared in mice with no ovarian tumors.

Except for an increase in ovarian tumors, 0.11 r did not seem to affect the ovaries, for no appreciable difference existed between the appearance of the ovaries of the controls and those of the irradiated mice. However, the tumor incidence in the controls of the present study was only 4 percent as compared with the 14 percent of the controls in the old experiment and 11 percent and 66 percent in the irradiated mice of the new and old experiment, respectively.

The lung-tumor incidence in this present study did not differ greatly from that found in the former one (when the occurrence in the males and females is combined) although the percentage of lung tumors in the new controls is larger than that previously found. One interesting observation might be made when the lung-tumor occurrence in the males and that in the females are considered separately. No sex difference was seen in the control animals, and no increase due to irradiation was seen in the females. A sex difference in the irradiated animals occurred, which was significant at the 2-percent level. This difference was due to an increase in lung tumors in the irradiated males, which is significant at the 5-percent level. It appears that this low-level exposure did increase the lung-tumor incidence in the males but not in the females.

The data presented here indicate a lower lung-tumor incidence than that reported by Heston (*14*), who found an incidence of 53 percent in LAF_1 mice 18 months of age. However, Heston examined the lungs for tumors under the dissecting microscope, whereas we did not use the microscope.

According to Metcalf and Inda (*15*) exposure of rats to X radiation doses of 0.1 to 10 r per day increased the incidence of leukemia and reticulum-cell sarcoma. Nevertheless, the increase found in the LAF_1 mice exposed to 0.11 r was not expected because only exposure levels of greater than 2.2 r caused an increase in lymphocytic neoplasms in the original experiments. The sex difference in the occurrence of lymphocytic neoplasms is the reverse of that found in LAF_1 mice exposed to the ionizing radiations of nuclear detonations where thymic lymphoma appeared more frequently in the males than in the females (*16*). Induction of these neoplasms by chronic irradiation may differ from that caused by a single exposure.

The increase in weight of the males is a typical response to chronic low-dose irradiation. There is no need to discuss it here, because it has been thoroughly covered in the report of the original experiments and no new data have been obtained to explain this observation.

Conclusion

LAF_1 male mice exposed to γ radiation of radium at a dose level of 0.11 r per 8-hour day from 1 month of age until death had a mean survival time of 783.1 days while that of nonirradiated LAF_1 male mice had a mean survival time of 683.5 days. This increase of 99.6 days is significant at the 0.01 level.

LAF_1 female mice similarly exposed had a mean survival time of 820.4 days as compared to 802.9 days of the controls. This increase of 17.4 days is not significant.

An increase in the incidence of lymphosarcomas and other tumors of the reticular tissues occurred in both the male and female irradiated mice. Mammary carcinoma incidence was also higher in the irradiated animals. The increase in both cases was statistically significant at the 0.01 level. In addition, the irradiated females showed an increase in ovarian tumors ($P<.05$) and the irradiated males showed an increase in lung tumor incidence ($P<.05$).

References

(*1*) LORENZ, E., and HESTON, W. E.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. I. Preliminary experiments. *In* Biological Effects of External X and Gamma Radiation, Part I. (Zirkle, R. E., ed.). National Nuclear Energy Series IV-22B. New York, McGraw-Hill, 1954, pp. 1–11.

(*2*) LORENZ, E.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. II. Physical arrangement. *In* National Nuclear Energy Series IV-22B. New York, McGraw-Hill, 1954, pp. 12–23.

(*3*) LORENZ, E., JACOBSON, L. O., HESTON, W. E., SHIMKIN, M., ESCHENBRENNER, A. B., DERINGER, M. K., DONIGER, J., and SCHWEISTHAL, R.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs, and rabbits. III. Effects on life span, weight, blood picture, and carcinogenesis and the role of the intensity of radiation. *In* National Nuclear Energy Series IV-22B. New York, McGraw-Hill, 1954, pp. 24–148.

(*4*) DERINGER, M. K., HESTON, W. E., and LORENZ, E.: Effects of long-continued total-body gamma irradiation of mice, guinea pigs, and rabbits. IV. Actions on the breeding behavior of mice. *In* National Nuclear Energy Series IV-22B. New York, McGraw-Hill, 1954, pp. 149–168.

(*5*) ESCHENBRENNER, A. B., and MILLER, E.: Effects of long-continued total-body gamma irradiation of mice, guinea pigs, and rabbits. V. Pathological observations. *In* National Nuclear Energy Series IV-22B. New York, McGraw-Hill, 1954, pp. 169–225.

(*6*) LORENZ, E.: Effects of long-continued total-body gamma irradiation of mice, guinea pigs, and rabbits. VI. Conclusions and applicability of results to the problem of human protection. *In* National Nuclear Energy Series IV-22B. New York, McGraw-Hill, 1954, pp. 226–242.

(*7*) BLAIR, H. A.: A formulation of the injury, life span, dose relations for ionizing radiations. I. Application to the mouse. UR-206 (Univ. of Rochester), 1952.

(*8*) LORENZ, E., DERINGER, M., and SCHWEISTHAL, R.: Progress report: Survival of mice following acute and chronic total-body x irradiation. Argonne National Laboratory, ANL-4625, 1951.

(*9*) LORENZ, E., SCHWEISTHAL, R., and CONGDON, C. C.: Progress report: Long-term survival of X-irradiated mice with and without spleen protection. Argonne National Laboratory, ANL-4948, Nov. and Dec. 1952 and Jan. 1953.

(*10*) DUNN, T. B.: Relationship of amyloid infiltration and renal disease in mice. J. Nat. Cancer Inst. 5: 17–28, 1945.

(*11*) HESTON, W. E., LARSEN, C. D., and DERINGER, M. K.: Variations in occurrence of pathologic calcification, nephritis and amyloidosis in mice fed control and modified diets. J. Nat. Cancer Inst. 6: 41–47, 1945.

(*12*) DUNN, T. B.: Some observations on the normal and pathologic anatomy of the kidney of the mouse. J. Nat. Cancer Inst. 9: 285–301, 1949.

(*13*) HESTON, W. E., and DERINGER, M. K.: Hereditary renal disease and amyloidosis in mice. Arch. Path. 46: 49–58, 1948.

(*14*) HESTON, W. E.: Inheritance of susceptibility to spontaneous pulmonary tumors in mice. J. Nat. Cancer Inst. 3: 79–82, 1942.

(*15*) METCALF, R. G., and INDA, F. A.: Pathology in animals subjected to repeated daily exposure to roentgen rays. UR-88, 1951.

(*16*) FURTH, J., UPTON, A. C., CHRISTENBERRY, K. W., BENEDICT, W. H., and MOSHMAN, J.: Some late effects in mice of ionizing radiation from an experimental nuclear detonation. Radiology 63: 562–569, 1954.

Long-Term Survival Following X Irradiation and the Irradiation of the α Particles from Radon and Its Decay Products[1]

JOANNE WEIKEL HOLLCROFT, EGON LORENZ, MARION MATTHEWS, *and* CHARLES C. CONGDON,
National Cancer Institute,[2] Bethesda 14, Md.

In a previous paper the 30-day LD_{50} of X ray was reported to be 1.42 times that of the α particles from radon and its daughters (*1*). The exposures were based on the total energy absorbed. It was also shown that the short-lived decay products concentrated in the kidney and there produced a dose 14 times the average dose. The radon and X-ray regression curves were not parallel, however, thus indicating the possibility of a difference in mode of death.

Dr. Thelma Dunn (*2*), while examining kidney sections of leukemic DBA mice treated with radon, pointed out the unique alteration in the histology of the cortical portion of kidneys. Tubular atrophy and bizarre nuclei in the tubular epithelium were observed. This finding, along with the discovery of the high kidney dose, pointed to renal failure as a cause of death. This had not been found after X irradiation.

Lacassagne (*3*) found that a rabbit after receiving polonium apparently died of severe kidney damage while a rabbit receiving 10 times the energy dose of X irradiation did not die.

Damage to the kidneys rarely occurs after doses of less than 2,000 r X irradiation (*4–6*), although 630 r total-body irradiation has been reported by Upton and Furth (*7*) to induce nephrosclerosis in mice 15 months after treatment. On the other hand, kidney damage has been reported following injection of polonium, plutonium, or radium (*4, 5*).

This study reports the survival time after a near-lethal dose of radiation by α particles from radon and its short-lived decay products, RaA, RaB, RaC and RaC', and a comparison is made with survival time following X irradiation with a comparable total-body exposure. In addition, a short report is made of the character of the kidney lesion.

Method

Strain A male mice derived from the Bittner subline but raised in this laboratory were used in these experiments. They were 3 to 3.2 months

[1] Received for publication October 26, 1954.
[2] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

of age at treatment and were housed in plastic cages on the same shelf for the entire period of observation with the exception of one group mentioned below. The mice were weighed periodically and allowed to live out their entire life span after the initial treatment. Autopsies were not performed on these first three groups of animals, as survival time only was desired.

One group of 20 mice was kept as control. The second group of 20 mice was injected intravenously with a 4-hour-old solution of 0.016 mc. per gm. radon in equilibrium with its decay products. This amount of radon had been found (*8*) to give a dose equal in absorbed energy to that produced by 400 r X rays. This dose is largely from the RaC^1 α particles. For two days after injection these mice were kept in the treatment room.

The third group of 20 mice was X-irradiated with 400 r. The X irradiation was obtained from a 186 KVP, 20 Ma. beam at a distance of 164 cm. and extended over a 2-hour period. The filters were increased throughout this time to simulate the radon dose rate.

Three additional groups of strain A male mice of the Heston subline were similarly treated, but were killed serially and autopsied. The kidneys were weighed and half of each kidney was fixed in 10-percent formalin and stained with Sudan IV. The other half of the kidneys and other organs were fixed in formalin-Zenker solution and stained with hematoxylin and eosin.

The schedule for killing the radon-treated animals was as follows: 1 per day for 7 days following treatment, then 1 every other day for 7 days, then 1 per week for 5 months. The X-irradiated animals were killed as follows: 1 per day for 6 days, then 2 per week for 2 weeks, and the remainder at 1- or 2-week intervals. The controls were also killed at 1- or 2-week intervals. The mice to be killed were selected at random, except for 10 mice in each group which were used for blood counts and therefore kept until the end of the experiment.

Because the short-lived decay products of radon disintegrate and form RaD, which eventually forms RaE and polonium, some chronic irradiation will take place if these products are retained in the body. It was therefore thought advisable to study the retention of RaD and estimate the dose from RaD, RaE and polonium.

Strain A male mice (of both Heston and Bittner sublines) were injected intravenously with solutions of RaD in 0.1-percent sodium citrate. The mice were killed at 2, 4, 24 and 48 hours and at 6, 8, 15, 98 and 225 days after injection.

The kidneys, lungs, liver, femurs, sternum and spleen were weighed, placed in flat-bottomed pyrex ashing dishes and ashed for 3 days at about 50° C. on the first day, 80° C. on the second day, and 100° C. on the third day. The ash, still in the pyrex dish, was then ground in methyl alcohol, the methyl alcohol evaporated and the sample counted. Methyl alcohol was chosen to prevent loss of ash in grinding because of its low boiling point.

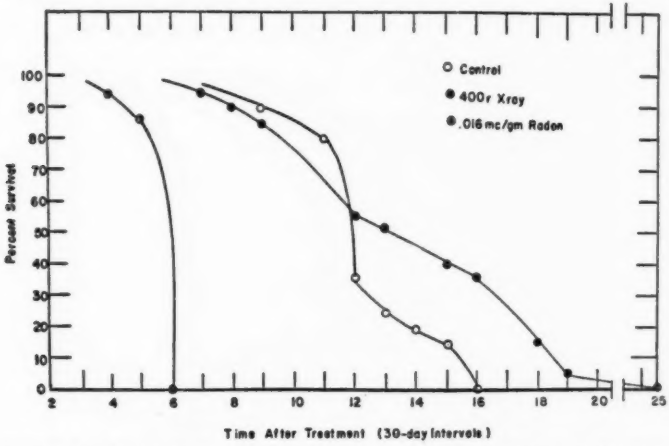
The carcasses were placed in weighed crucibles and ashed in a manner similar to that of the tissues, except the ashing time was at least twice

as great at each temperature. The total ash was weighed, and ground in a mullite mortar. Methyl alcohol was again employed. Suitable amounts of ash were transferred to metal counting dishes. The methyl alcohol was evaporated and the ash weighed.

When RaD was in equilibrium with RaE, the β particles from RaE were counted with a thin window-counter and the amount of RaD was determined. A correction for the difference in backscatter from glass to metal was made. The amount of energy absorbed by the tissues from RaD, RaE and polonium was determined by the method of Cornfield (*9*). To give a maximum energy, it was assumed that RaE and polonium were not eliminated.

Results

Several mice died from acute irradiation injury and were discarded. The radon treatment shortened the mean survival time by 56 percent but the 400 r X irradiation did not shorten the life span, as shown in table 1 and text-figure 1. Both the survival curves and the table show survival time after treatment. The animals X-irradiated with 400 r lived longer than the controls.



TEXT-FIGURE 1.—Survival curves of strain A male mice following: *a*) No treatment; *b*) 400 r X irradiation; or *c*) intravenous injection of 0.016 mc. per gm. radon in equilibrium with its daughters.

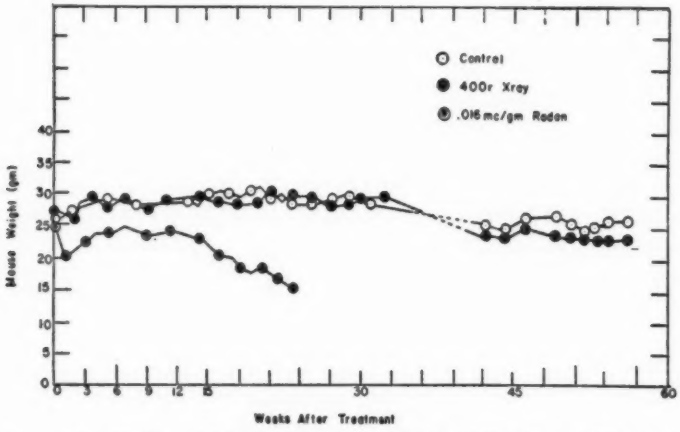
TABLE 1.—*Mean survival time of strain A male mice*

Experimental group	Mean survival time (*days after treatment*)	Decrease over controls (*percent*)	Significance of decrease
Control	377. 2 ± 17. 7*	.....	.....
0.016 mc./gm. Rn	165. 1 ± 3. 9	56. 2	$P<.01$
400 r X ray	425. 2 ± 29. 7	−12. 7	none

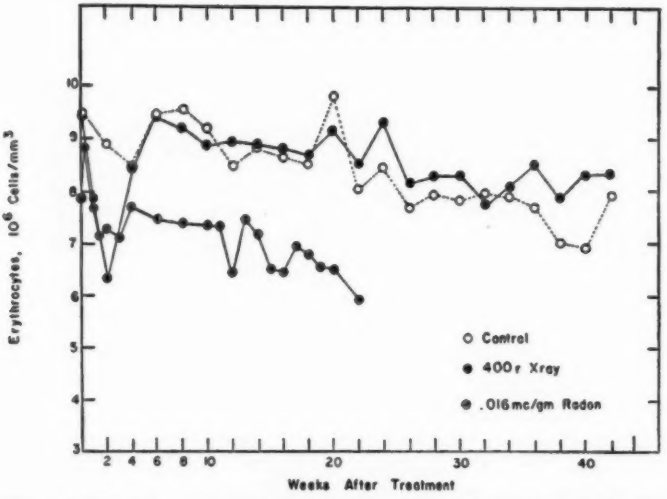
*Standard error.

The radon-treated animals lost weight after treatment and although they regained weight in 4 or 5 weeks, their weight never rose to control level (text-fig. 2). The X-irradiated mice lost very little weight, however, and remained at the control level throughout their life.

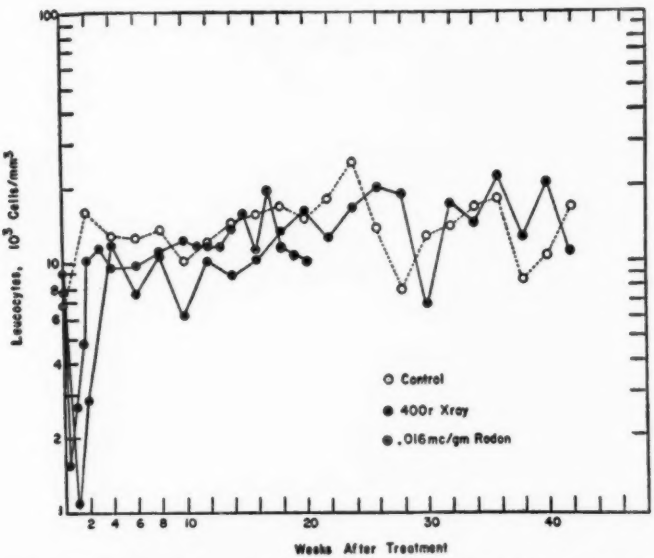
The erythrocyte counts of both the X-irradiated and the radon-injected mice dropped 2 weeks after treatment and began recovering 4 weeks after treatment (text-fig. 3). The radon-treated mice did not recover



TEXT-FIGURE 2.—Body weight of strain A male mice following: *a*) No treatment; *b*) 400 r X irradiation; or *c*) intravenous injection of 0.016 mc. per gm. radon in equilibrium with its daughters.



TEXT-FIGURE 3.—Erythrocyte count of strain A male mice following: *a*) No treatment; *b*) 400 r X irradiation; or *c*) intravenous injection of 0.016 mc. per gm. radon in equilibrium with its daughters.



TEXT-FIGURE 4.—Leukocyte count of strain A male mice following: *a*) No treatment; *b*) 400 r X irradiation; or *c*) intravenous injection of 0.016 mc. per gm. radon in equilibrium with its daughters.

completely; the red blood count remained below the level of the controls and dropped even further as the mice reached the end of their life span. No marked anemia was seen terminally in the animals treated with 400 r X ray. There was marked leukopenia and lymphopenia following treatment and this was of the same severity in both irradiated groups (text-fig. 4). The radon-treated mice showed a return to a normal leukocyte count sooner than the X-irradiated mice.

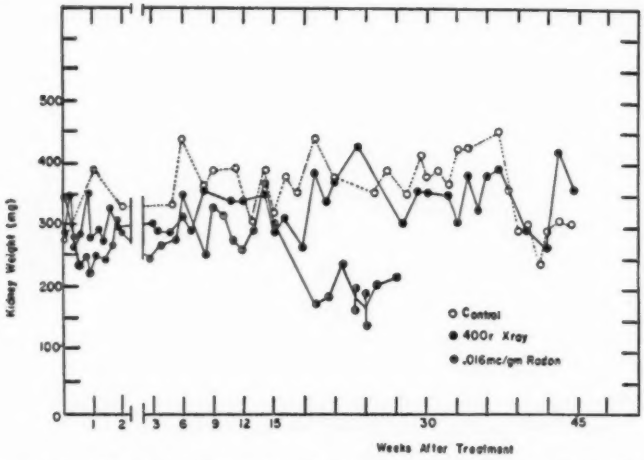
The kidney weights of the mice treated with 400 r X irradiation did not differ from those of the controls, but those of the radon-treated animals dropped 18 weeks after treatment (text-fig. 5). At autopsy these kidneys were contracted, very firm in consistency, and gray-brown in color.

The histologic study of the kidneys in the three groups revealed scattered cases of pyelonephritis both active and healed, papillonecrosis and amyloidosis in the control group. In the group that received 400 r total-body X irradiation the kidneys resembled those seen in the untreated controls. These inflammatory kidney lesions appeared only in mice more than 8 months old. Of the 18 control animals killed 20 weeks or more after start of the experiment, 11 showed inflammatory kidney lesions, while they appeared in only 4 of the 17 X-irradiated mice killed in the same period.

The animals injected with radon showed (beginning 6 weeks after treatment) extreme atrophy of the cortex. Occasional convoluted tubules had a relatively normal appearance but the majority showed

marked distortion of the lining cells with atrophy a prominent feature (fig. 1). Many bizarre giant nuclei were present. Some of the glomeruli showed thickening of the capillary walls and increased endothelial cells but the majority showed a normal appearance (fig. 2).

In the first week after treatment the hematopoietic organs of both treated groups were essentially the same and showed acute radiation changes. The spleens of the radon-treated animals began regeneration earlier than those of the X-irradiated animals. In addition, in the first 9 days after radon injection, kidney damage consisting of degenerative fatty infiltration of the tubular epithelium was seen throughout the cortex in several mice.



TEXT-FIGURE 5.—Kidney weights of strain A male mice following: *a*) No treatment; *b*) 400 r X irradiation; or *c*) intravenous injection of 0.016 mc. per gm. radon in equilibrium with its daughters.

At all periods later than 2 weeks following treatment when animals of comparable age were killed, all organs except the kidneys appeared histologically similar. The only exception was a radon-treated animal killed 21 weeks after treatment in which a lymphosarcoma had replaced the thymus.

The chronic radiation produced by RaD, RaE and polonium was very small. RaD was eliminated very slowly from the mouse (table 2). However, the amount in the lungs, liver and spleen became negligible by the 15th day. The content of the kidneys was relatively high and dropped gradually until at 98 days it was undetectable, by the methods used. On the other hand, the content of the femur rose during the first day and remained relatively constant for 2 weeks and then was eliminated extremely slowly. The content of the sternum was of the same order of magnitude as that of the femur. The spread in data was considerable, and the data represent only 4 animals at each time interval.

TABLE 2.— *Radium D retention*

Time (*days*)	Whole body	Kidney	Femur
	RaD / RaD injected	RaD/gm. kidney / RaD injected	RaD/gm. femur / RaD injected
0	1. 00	.....	.....
1/12	.....	0. 63	0. 065
1/6	0. 93	.....	. 081
1	. 86	. 21	. 144
2	. 56	. 45	. 118
6	. 41	. 12	. 150
8	. 32	. 15	. 140
15	. 25	. 03	. 132
98	. 19	0	. 082
225	. 089	.....	. 080

The total energy absorbed in the mouse from RaD, RaE and polonium when 0.016 mc. per gm. of a 4-hour-old solution of radon was injected was estimated to be 599 ergs per gm. or 6.4 r (93 ergs per gm. equals 1 r). The total energy absorbed in the kidney was estimated to be 1,550 ergs per gm. or 17 r, and in the sternum 5,157 ergs per gm. or 55 r, provided RaE and polonium are not eliminated.

Discussion

RaB, RaC and RaC[1] have previously been found to concentrate in the kidneys, and the radiation dose to the kidney has been estimated to be 520,000 ergs per gm. following an intravenous injection of 0.016 mc. per gm. radon in equilibrium with its decay products (*1*). It is, then, not surprising to find that earlier death in animals following administration of radon and its decay products can be explained by renal failure. This is probably due to renal failure which may also account for the terminal anemia and weight loss.

The kidney damage appears to be the same as that described by Cassarett (*5*) in rats receiving polonium injection but different from the X-ray-induced nephrosclerosis of Upton and Furth (*7*). Cassarett observed the first changes in rat kidneys 28 days after polonium injection and marked changes at 42 days. The mouse kidneys showed an early damage in the first week and then appeared normal until the 42nd day.

More mice are required in order to evaluate the extent of the early degenerative changes, from which the animal apparently recovered. Injuries which produce such profound later changes, after radiation of 2-hour duration, should be visible. This early kidney injury may have increased the number of early deaths after near lethal doses. (In the 30-day LD_{50} study, the radon-treated animals died earlier than the X-irradiated animals, even though the white counts returned to normal sooner.) If a more even distribution of radon-decay products were obtained, perhaps a higher LD_{50} dose would be found. On the other hand, the relative biological effectiveness of α particles cannot be compared with

that of X rays by comparing LD_{50} doses unless the cause of death is known to be the same.

The late kidney damage cannot be the result of the RaD, RaE, or polonium remaining in the kidney for these produce only slight radiation energy over the entire life span. Furthermore, the damage cannot be attributed to heavy metal toxicity because there is much less than 10^{-11} gm. RaD, per gm. kidney, and also because bismuth and lead isotopes would not be expected to cause the damage seen here.

Between the tenth and eleventh month after treatment, the air-conditioning in the animal room broke down and the room was very warm for several days. During this time 4 mice in the X-irradiated group and 7 in the control group died; hence the sudden dip in the survival curve.

Although the number of animals in the life span study is too small to show a real increase in mean survival time following 400 r, the results suggest that 400 r does not shorten the life span of strain A males.

This is not in agreement with survival data found on C3Hf mice where an acute dose 400 r X irradiation reduces the mean survival time from about 700 days to 510 days (*10*). Strain A male mice X-irradiated with 400 r at birth in this laboratory (*11*) showed an increase in lymphosarcoma incidence at 1 year. On the other hand, the serial killing experiments indicate that inflammatory kidney lesions were more prevalent in the control animals than in the irradiated mice. This suggests that the short life span of the controls is caused by these inflammatory lesions.

Summary

Strain A male mice were treated with 400 r X irradiation, injected with a dose of radon in equilibrium with its daughters such that the absorbed energy was equivalent to that of 400 r, or kept as controls. The mean survival time of the X-irradiated animals was found to be 425, that of the radon-treated mice 165 days, and that of the controls 377 days.

A small serial killing study was done on mice treated similarly that showed that the shortened life span following radon treatment could be explained by renal damage, which was not found in mice of the other groups. An early kidney damage was also seen in the week following radon injection.

In addition, data obtained on the storage of RaD in the body indicated that RaD remained in the femur, sternum and kidneys, although it was eliminated from other tissues relatively rapidly. The kidney dose from RaD, RaE, and polonium after injection of 0.016 mc. per gm. of radon in equilibrium with its short-lived decay products was estimated to be 1,550 ergs per gram or 17 r.

These findings indicate that the kidney damage caused by a high dose of irradiation from α particles may be in part responsible for acute deaths following radon administration. Since this damage is not seen following X irradiation in the LD_{50} range, the calculation of relative biological effectiveness based on an LD_{50} dose of radon as compared to X ray is not valid.

References

(1) HOLLCROFT, J. W., and LORENZ, E.: The 30-day LD-50 of two radiations of different ion density. J. Nat. Cancer Inst. 12: 533–544, 1951.

(2) DUNN, T.: Personal communication, 1945.

(3) LACASSAGNE, A.: Les cancers produit par les rayonnements corpusculaires: Mécanisme présumable de la cancérisation par les rayons. IV. Radiophysiologie expérimentale, cancers et hormones, exposés publiés sous la direction de Antoine Lacassagne. Actualités scientifiques et industrielles, 981. Paris, Hermann and Cie, 1945, pp. 67–68.

(4) BLOOM, W.: The kidney. *In* Histopathology of Irradiation (Bloom, W., ed.). National Nuclear Energy Series IV-22I. New York, McGraw-Hill, 1948, pp. 674–676.

(5) CASSARETT, G. W.: The kidney. *In* Histopathology of Alpha Radiation from Internally Administered Polonium. UR-201 (Univ. of Rochester) Part II, 1952.

(6) WARREN, S.: Effects of radiation on normal tissues. VII. Effects of radiation on the urinary system. Arch. Path. 34: 1079–1084, 1942.

(7) UPTON, A. C., and FURTH, J.: Nephrosclerosis induced in mice by total-body irradiation. (Abstract.) Fed. Proc. 13: 445, 1954.

(8) HOLLCROFT, J. W., and LORENZ, E.: Retention of radon by the mouse. I. Nucleonics 5: 63–71, 1949.

(9) CORNFIELD, J.: Retention of radon by the mouse. II. Nucleonics 5: 68–75, 1949.

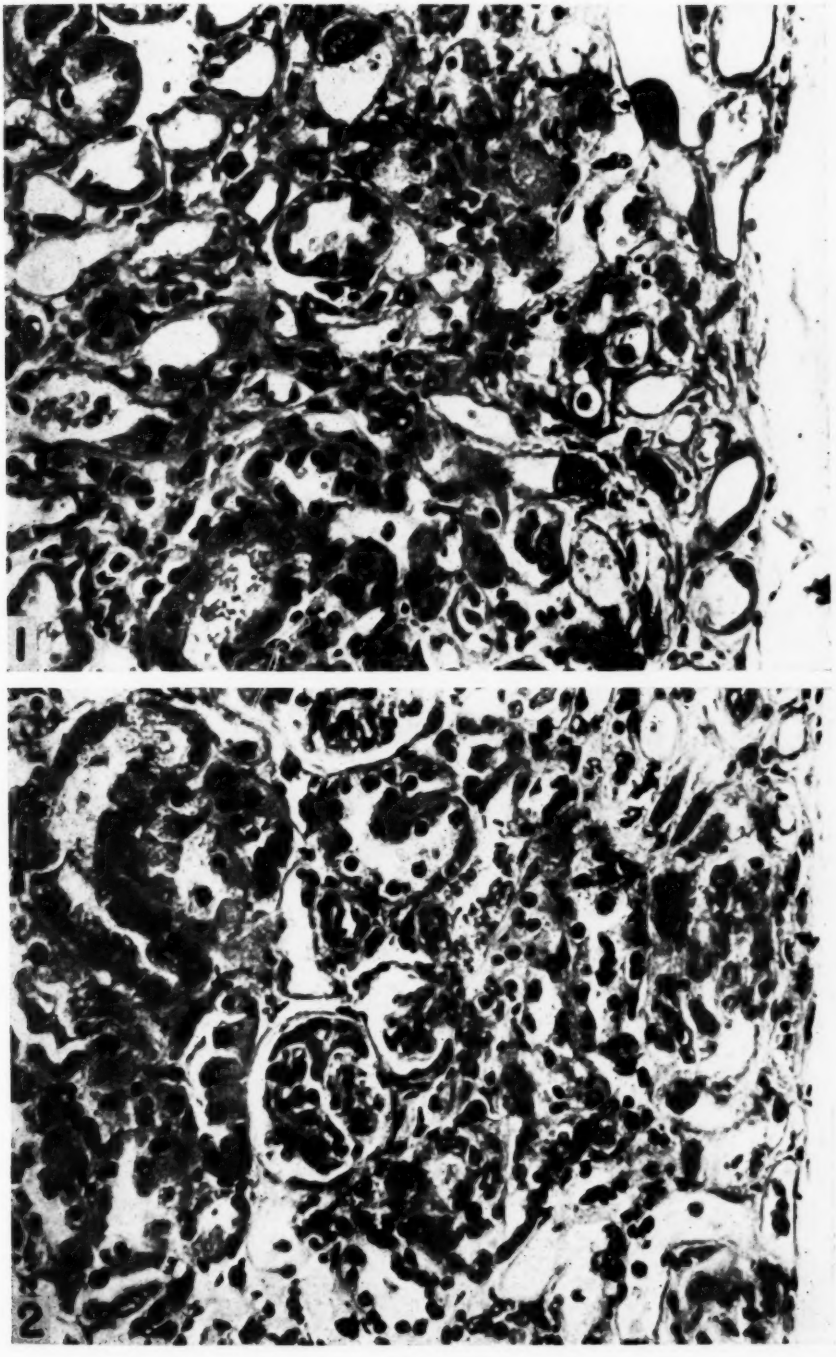
(10) LORENZ, E., SCHWEISTHAL, R., and CONGDON, C. C.: Progress report: Long-term survival of X-irradiated mice with and without spleen protection. Argonne National Laboratory, ANL-4948, Nov., Dec. 1952, and Jan. 1953.

(11) LORENZ, E., and DUNN, T. D.: Ocular lesions induced by acute exposure of the whole body of newborn mice to roentgen radiation. Arch. Ophth. 43: 742–749, 1950.

PLATE 65

FIGURE 1.—Irregular atrophy and atypical hyperplasia of cells lining the convoluted tubules of the kidney of a radon-injected mouse killed and autopsied 140 days after injection. Hematoxylin and eosin. × 340

FIGURE 2.—Photomicrograph of another area from the same kidney shown in figure 1. Note the abnormal glomerulus in addition to the tubular changes. An arteriole with a thickened wall is present above the glomerulus. Hematoxylin and eosin. × 340



320174—55——23

Experiments on the Disposition of Acutely Inhaled $C^{14}O_2$[1]

AUSTIN M. BRUES *and* AGNES N. STROUD, *Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.*

Previous reports (*1*, *2*) have dealt with experiments on the long-term results following a short period of inhalation of $C^{14}O_2$. This report is concerned with the fate of the C^{14} absorbed during a 30- to 60-minute period of exposure to $C^{14}O_2$.

Materials and Methods

Male Sprague-Dawley rats, weighing 200 to 250 gm., and female CF-1 mice, weighing 18 to 30 gm., were used in these experiments. Tracheal cannulae were inserted under nembutal anesthesia. A simple valve system, constructed in a lucite block by Mr. George Svihla, was placed distal to the cannula so that air was inhaled from the room or from a vessel saturated with water vapor containing $C^{14}O_2$ and exhaled into a system to which rubber balloons were attached for the collection of samples. The measured dead space in the apparatus used for mice was less than 0.1 cc. and in that for rats was between 0.3 and 0.4 cc.

Counting of air samples was done by admitting 50-cc. samples (or measured samples of smaller volume) from an oiled syringe into a partly evacuated 700-cc. chamber containing a thin-window Geiger-Muller tube, which was then brought to atmospheric pressure by the rapid admission of room air. Exhaled air was thus compared directly with inhaled air independently of CO_2 concentration. Although the counting yield was less than 0.2 percent, this method had certain obvious advantages for the rapid counting of samples of $C^{14}O_2$ with widely variant CO_2 concentrations.

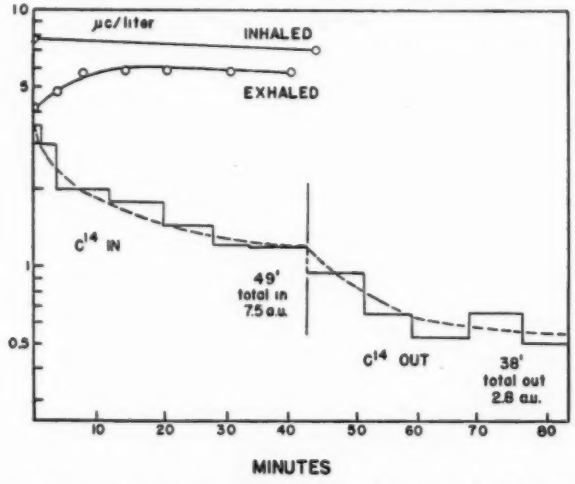
Consideration was given to the effect on counting of the changed gas composition of the air as it was inhaled and exhaled. The change in density can be considered negligible (less than 0.2 percent under the conditions specified), and if saturated water vapor is inhaled the only change in volume through the respiratory cycle would be due to the effect of a respiratory quotient less than unity, which would introduce a correction not exceeding 1 percent. Since the counting chamber was very large with respect to the range of C^{14} β rays, the effect of changing barometric pressure and temperature would likewise be negligible.

[1] Received for publication November 5, 1954.

After permitting the anesthetized animals to breathe room air through the apparatus, a valve was turned admitting air containing the tracer. Expired air was collected in ordinary rubber balloons, and it was ascertained that no measurable loss of C^{14} occurred from these balloons over a period of 3 to 4 hours. All expired air was collected at intervals of 1 to 8 minutes. After the exposure period the intake stopcock was again switched to receive room air, and collections of expired air were continued for about 1 hour. Since the room air was not specially humidified, it can be estimated that the readings on expired air during this terminal period should be corrected upwards by about 2 percent. The experiments usually had to be terminated within 2 hours after cannulation because of the collection of mucus in the cannulae.

Results

Text-figure 1 illustrates the results of a typical mouse experiment. The two upper lines show the concentration of C^{14} in inhaled and exhaled air during the exposure period. The lower lines show the rate of accumulation of C^{14} in the mouse and (after the vertical line) the rate of loss from the animal after changing over to room air. The fact that the rate of loss just after the end of exposure and the rate of intake just before are equal is fortuitous and simply means that at that time the specific activity of blood bicarbonate was about half that of alveolar CO_2.



TEXT-FIGURE 1.—Accumulation and loss of C^{14} during and after exposure of a mouse to $C^{14}O_2$. The *upper* lines indicate the level of $C^{14}O_2$ in μc. per liter in inhaled and exhaled air. The *lower* solid line indicates the rate (amount per minute in arbitrary units, a.u.) of accumulation before termination of exposure (at vertical line) and the rate of loss, in the same units, after termination. *Broken* lines are smoothed rates based on the actual measurements represented by the *solid* lines.

Interpretation of data thus gained from the experiments makes it possible to state certain facts:

1) Percent clearance of C^{14} from the air is shown by the rate of C^{14} uptake extrapolated back to zero time, where C^{14} is first introduced into the inhaled air. This value is usually between 50 and 65 percent. The major factor maintaining this value below 100 percent is the dead space which, calculated from CO_2 content of expired air assuming an alveolar CO_2 of 5.5 percent, is 50 percent in the mouse and 33 percent in the rat. An additional factor is due to the net loss of CO_2 from blood during the respiratory cycle, which requires that about 10 percent of the amount of $C^{14}O_2$ taken into the alveoli shall remain there at the time of expiration.

2) The rate of C^{14} uptake decreases rapidly at first as the blood and tissue bicarbonate become saturated with the isotope. After about 20 minutes in the mouse and 30 to 35 minutes in the rat, a phase is reached in which the uptake becomes almost constant. In this period the major factor in the uptake of C^{14} is its net movement into other compartments, presumably organic compounds and bone carbonate.

3) The rate of C^{14} loss after the termination of the exposure period is considerably less than the initial uptake rate. The specific activity of expired CO_2 at this time must be equal to that of venous blood bicarbonate, and from this and the plateau uptake of C^{14}, one can calculate an approximate value for the rate at which carbon exchanges between blood bicarbonate and the inner compartments.

4) The size of these inner compartments cannot be determined directly from these acute experiments. This can be found by long-term inhalation experiments, since they tend to reach a constant activity after several days of exposure.

The following tables give values derived from the experiments. Two assumptions have been made in calculating these values: that the alveolar CO_2 concentration is 5.6 percent (2.5 m*M*/liter) and that the total-body bicarbonate-CO_2 content is 12 m*M* per kg. Data on intake of C^{14} represent the average of values for 5 mice and 11 rats. Three of the rats received 5 percent CO_2 throughout the experiment, but none of the results differed significantly from those in rats breathing normal air.

In table 1 the uptake of CO_2 from alveolar air into blood is shown to be of the same order of magnitude as the loss in the opposite direction. This might be anticipated, since CO_2 exchanges readily between blood and alveoli and since the concentration of bicarbonate in blood is much higher than that of CO_2 in the alveoli. The rate of uptake by tissue organic compounds, being some fraction of the rate of passage into inner compartments is, however, less than one-half of the rate of net CO_2 loss. The turnover rate between blood and the inner compartments is 40 percent of that between alveolar air and blood in the mouse, and 49 percent in the rat.

Table 2 shows the fate of the C^{14} passing into the inner compartments during the 45-minute exposure. The total C^{14} space in terms of μM of alveolar CO_2 is shown first; from this and the blood specific activity is calculated the amount of the absorbed C^{14} that is in blood and tissue bicarbonate and the amount in the inner compartment.

TABLE 1.—*Respiratory exchange of C^{14}*

	Mice		Rats	
	Mean	Range	Mean	Range
Weight (gm.)	25	18–30	232	204–248
Minute volume (cc.)	16. 1	10–21	100	75–130
Tidal air (cc.)	0. 15	0. 1–0. 22	1. 5	1. 4–1. 6
Dead space (cc.)	0. 06	0. 04–0. 08	0. 5	0. 4–0. 6
Initial C^{14} clearance (%)	55. 7	50–61	62. 5	50–76
C cleared from alveolar air to blood, μM/min	24	13–30	161	142–200
Apparent amount cleared through to inner compartments	5. 6	4. 5–7. 5	45	25–55
C^{14} saturation of venous blood at 45 min. (%)	58	51–75	56	45–66
C cleared from blood to inner compartments, μM/min	9. 9	6. 7–14. 7	79	55–98

TABLE 2.—*Distribution of C^{14} after a 45-minute exposure period*

	Mouse	Rat
(a) C^{14} space as mM of alveolar CO_2	0. 35	2. 4
(b) Specific activity of blood, as % of alveolar	58	56
(c) Assumed blood and tissue bicarbonate, mM	0. 30	2. 8
(d) C^{14} space in blood and tissue bicarbonate (b × c)	0. 174	1. 57
(e) C^{14} space in inner compartments (a − d)	0. 176	0. 83
(f) Percent of absorbed C^{14} in inner compartments (e/a)	50	34

Thus, after a 45-minute exposure, a considerable portion of the absorbed CO_2 is in forms other than bicarbonate ions. At the end of exposure, it is probable that C^{14} passes from the intermediate bicarbonate compartment in both directions (toward alveoli and toward inner compartments) until its specific activity becomes reduced to the same level as that of the more labile inner compartments. Inspection of certain of the mouse experiments indicates that a reversal of flow occurs when the inner compartments contain about 3/4 of the retained carbon, indicating that the order of magnitude of the more labile components is about three times that of the bicarbonate pool, or 1 mM in the mouse. Long-term exposures indicate a somewhat larger compartment exchangeable with CO_2 (*3*).

Table 3 shows the distribution of C^{14} after 45 minutes of exposure, expressed in terms of the volume of inhaled air from which the C^{14} has

TABLE 3.—*Distribution of C^{14} after a 45-minute exposure period**

	Mouse	Rat
Air cleared (cc.)	141	836
Amount in inner compartments	71	280
Amount per gram	2. 84	1. 21
Organic C^{14} per gram liver	.....	2. 24
$C^{14}O_2$ per gram bone	.....	3. 65
Amount lost in 1 hour following exposure period	67	419

*Note: all values in cc. of air equivalent.

been cleared. In addition, the results of analyses of bone for carbonate and of liver for organic carbon are shown (mean values of 3 rats).

The nature of the organic C^{14} compounds developed in liver at this time is not known; analyses of alcohol-soluble and alcohol-insoluble fractions showed that C^{14} was about equally distributed between them. In three experiments, the alcohol-soluble moiety made up 46, 50, and 60 percent of the total. The composition of fixed carbon at its saturation value has been studied in detail (*4*).

It may be interesting to consider the rate of passage of C^{14} into organic carbon of liver and into bone carbonate in this brief period in relation to the rate at which it later approaches saturation. Calculations made from the data in table 3 have been compared with the figures given by Buchanan and Nakao (*3*, *5*). If we assume that the carbon of liver is about 14 m*M* per gm. (*2*) and that about 2 percent of this is eventually saturated with air-derived carbon (*3*), and if we take the alveolar air to be 2.2 m*M* per liter, it can be estimated that the organic carbon of liver has reached 1.8 percent of its saturation value in 45 minutes, while in one day those figures which have been published (*3*) suggest that it has reached about one half of the saturation value. A similar calculation for bone carbonate shows that it has exchanged to the extent of 1 percent with alveolar CO_2 in 45 minutes, and about 17 percent at the end of a day (*5*). In spite of the fact that these figures are derived from different sources, and the nature of some of the assumptions, it does appear that the incorporation of C^{14} from CO_2 into tissue and bone proceeds at a relatively steady rate until it approaches its saturation value.

Summary

1) Clearance of $C^{14}O_2$ from air into the blood of rats and mice proceeds with almost theoretical efficiency. Calculated rates of passage of alveolar CO_2 into the blood are 24 μM per minute in the mouse and 160 μM per minute in the rat. Uptake of C^{14} is essentially unaffected by the CO_2 concentration of inhaled air.

2) Passage of C^{14} from the bicarbonate compartments into inner compartments proceeds at a somewhat slower rate: 10 μM per minute in the mouse and 80 μM per minute in the rat. At the end of a 45-minute exposure, the specific activity of venous blood is 55 to 60 percent that of alveolar air.

3) After a 45-minute exposure, one half of the absorbed C^{14} in the mouse and one third in the rat, have passed through the bicarbonate pool into inner compartments. These include bone carbonate and both alcohol-soluble and alcohol-insoluble organic compounds in tissue.

Literature Cited

(*1*) Brues, A. M., and Naranjo, A.: Absorption and excretion of carbon 14 as CO_2 and bicarbonate. (Abstract.) Fed. Proc. 8:18, 1949.

(*2*) Brues, A. M., and Stroud, A. N.: Retention of C^{14} administered as bicarbonate to adult and embryonic mice. *In* Biological Hazards of Atomic Energy (Haddow, A., ed.). Oxford, Clarendon Press, pp. 171–180, 1952.

(3) BUCHANAN, D. L.: Uptake and retention of fixed carbon in adult mice. J. Gen. Physiol. 34: 737–759, 1951.
(4) SWICK, R. W., BUCHANAN, D. L., and NAKAO, A.: The normal content of fixed carbon in amino acids. J. Biol. Chem. 203: 55–61, 1953.
(5) BUCHANAN, D. L., and NAKAO, A.: Turnover of bone carbonate. J. Biol. Chem. 198: 245–257, 1952.

Effects of Combined Local Treatment With Radioactive and Chemical Carcinogens [1]

ARTHUR M. CLOUDMAN,[2] KATHERINE A. HAMILTON, RALPH S. CLAYTON,[3] *and* AUSTIN M. BRUES,
Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.

It is becoming generally appreciated that carcinogenic agents are of universal occurrence and there is reason to believe that the environmental hazard which they represent is increasing. In view of this, and of the complexity of the carcinogenic process in terms of the existence of cofactors and of stages in the development of induced malignant tumors, it is obviously of interest to study the interaction of radioactive and chemical carcinogens. For this reason, a series of experiments was set up to test the interaction between methylcholanthrene and agents emitting *beta* radiations in producing tumors of skin and underlying tissues.

Materials and Methods

CF-1 female mice, obtained from Carworth Farms, were employed in these experiments. The mice were from 42 to 55 days of age and weighed between 18 and 23 grams. After treatment as described below, they were kept under observation for the duration of life and sacrificed when moribund. Pathologic examination was performed routinely, including sections of tumors and other grossly abnormal tissues. Two experiments were done—one involving superficial applications, the other implantation of pellets.

Superficial carcinogenesis.—In this experiment, methylcholanthrene was applied by the use of a #4 camel's hair brush. Three brush strokes were used, covering an area about 4 × 1 cm. on the dorsal surface, centering around the midline and extending from scapulae to pelvis. An examination of the amount of methylcholanthrene involved was made by measuring the depletion rate of a 0.6-percent solution of methylcholanthrene in benzene during painting; it was found that the three strokes delivered an average of 0.29 ml. of benzene containing 1.74 mg. of the carcinogen. This was the usual dose; in one series one tenth of this concentration was applied. The amount of carcinogen solution delivered in these experiments

[1] Received for publication November 5, 1954.
[2] Work on these experiments was nearly completed before Dr. Cloudman's death.
[3] *Present address:* Radiologist, Parkland Memorial Hospital, Dallas, Texas.

was somewhat less than in the single-dose experiments of Cramer and Stowell (*1*).

Beta radiation was applied by the use of bakelite plaques containing P^{32} (*2*).[4] The mice were sedated by intraperitoneal injections of 1 mg. nembutal per gm. of body weight, confined by strings to a cork board, and exposed for the required length of time to the plaque, which was placed in a specially constructed lucite holder and arranged so that the plaque came in contact with a 4 × 1 cm. area of the mouse's back corresponding to that painted. The first plaque was estimated to yield 19,200 rep per hour at the time of receipt. This plaque was used until the dose rate had decayed by 53 percent, and a second plaque was employed, yielding 16,850 rep per hour and decaying by 26 percent in the course of exposures.[5] The durations of the individual exposures varied between 13.2 and 65.4 minutes depending on the dose required and the extent of the decay of the P^{32}. When both carcinogens were employed, the radiation was administered before the chemical exposure.

Two series of controls were used; 30 untreated mice, and 30 mice painted with the benzene used as solvent. Three groups of 30 were given applications of *beta* rays in the amounts of 5,000, 7,500, and 10,000 rep. A smaller group of 15 received 5,000 rep followed by painting with benzene. Methylcholanthrene (1.7 mg.) was administered alone and after 3,000 and 5,000 rep and 0.17 mg. methylcholanthrene was given after 5,000 rep (30 mice in each group).

Pellet exposures.—These exposures were accomplished by implantation of pellets in which the agents were incorporated in cholesterol. The pellets were prepared by drawing molten cholesterol (<185° C.) into capillary tubes of about 1-mm. caliber. For carcinogenic exposures, 1 percent methylcholanthrene in cholesterol was handled similarly, and radiation exposures were made by incorporating Y^{91} in the form of yttrium nitrate into the molten material. The pellets weighed, on the average, 3 mg., and those containing Y^{91} were planned to contain three amounts varying by factors of 10. Measurements made on the prepared pellets indicated that the mean values were 1.08, 10.8, and 72.6 μc., subject to 10-percent variation in either direction. Pellets were injected by trocar into the lower right back, through a small incision near the base of the tail.

In this experiment also, the two carcinogens were used alone as well as together. Three control groups, of 30 mice each, included untreated mice, those injected with cholesterol pellets, and those with pellets containing stable yttrium in the same concentration as the yttrium in the highest level of Y^{91}. Other groups were treated with pellets containing methylcholanthrene alone, and Y^{91} at the three concentrations used, with and without methylcholanthrene. Owing to accidental loss of some of the mice in the latter groups soon after injection, these groups are of variable size.

---

[4] The writers are indebted to Dr. G. E. Stapleton of Oak Ridge National Laboratory, who provided the plaques.

[5] The surface rates are based on extrapolation chamber measurements supplied with the plaques. Further measurements were made in this laboratory by extrapolation methods, and they may be taken as approximately correct

Results

Superficial exposures.—Loss of hair occurred after 80 to 100 percent of the exposures with either carcinogen. It took place much more rapidly after methylcholanthrene treatment than after irradiation alone (see table 1). In mice treated with methylcholanthrene alone, however, it was less persistent, and after 24 days 75 percent of the mice had shown regrowth; this was followed by a secondary transitory phase of epilation between 40 and 55 days. Twenty to twenty-five percent of those mice remained partially epilated throughout the experiment.

Mice treated by *beta* radiation alone (5,000 to 10,000 rep) remained, with isolated exceptions, epilated throughout life, but with a transitory recovery around 40 days after exposure. Those given 5,000 rep *beta* radiation plus methylcholanthrene showed a later transitory recovery between 100 and 125 days. Those given methylcholanthrene plus 3,000 rep were intermediate between the latter and mice treated by the chemical alone. This suggests a complicated interaction between the growth-inhibiting actions of the two agents.

Ulceration, when it occurred, also appeared sooner with methylcholanthrene, and healed sooner. Benzene alone, painted on the skin after *beta* irradiation, accelerated these responses somewhat, so that it is by no means certain that methylcholanthrene was responsible for acceleration of the *beta*-ray ulceration and its healing (benzene alone, however, produced no epilation or ulcers). Papillomatosis also appeared earlier after methylcholanthrene than after *beta*-ray application or a combination of the two; however, a synergism in respect to multiple papillomatosis is suggested by the data. Thus, the total number of papillomas seen up to 8 months after treatment exceeds the number of mice where methylcholanthrene and *beta* rays were combined (1.7 mg. + 5,000 rep) and they are less than one fourth as numerous when either treatment was given alone. These observations are tabulated in table 1.

When the survival curves of the painting experiments are examined (table 2), the remarkable fact is noted that methylcholanthrene at the higher dose level causes a considerable reduction in survival, while *beta* radiation, although almost as carcinogenic, has no measurable effect on survival.

Autopsy data were examined in an attempt to see if there was a single explanation for the decreased survival following a single methylcholanthrene application. It was noted that the incidence of pulmonary tumors is increased in these experiments, from 70 percent in those not receiving methylcholanthrene to 91 percent in those receiving it. It seems likely, however, that this cannot alone explain the difference, since survival was also shortened in those mice that did not have pulmonary tumors.

The production of malignant tumors is summarized in table 3. These data make it appear that the effects of the two agents are approximately additive. We note that subcutaneous tumors appear only when radiation has been applied, and that tumors appear earlier in those animals treated with methylcholanthrene. It is possible that the chemical agent may

TABLE 1.—*Time and incidence of epilation, ulceration and papilloma formation in superficially treated mice*

	P^{32} 5,000 rep	P^{32} 5,000 rep + benzene	P^{32} 7,500 rep	P^{32} 10,000 rep	MCA 1.7 mg.	MCA 1.7 mg. + 3,000 rep	MCA 1.7 mg. + 5,000 rep	MCA 0.17 mg. + 5,000 rep
Days to maximum epilation (80–100 % of mice)	22	16	24	22	6	11	11	13
Days to maximum ulceration (50–90 % of mice)	22	16	24	22	10	15	16	15
Days to healing of ulceration in all mice	107	40	104	73	24	105	40	39
Maximum % of mice with early papillomas	20	13	10	7	17	37	30	10
Days to maximum early papilloma formation	82	61	68	69	45	70	80	74
Cumulative % of mice with papillomas up to 250 days	10	20	17	7	17	46	70	20
Mean number of tumors per mouse with papillomas	1.0	1.8	1.0	1.4	1.6	1.4	2.4	1.2

TABLE 2.—*Days to various percentages of survival (skin applications)*

Percent survival	Controls	Benzene	P^{32}				MCA 1.7 mg.			MCA 0.17 mg.
			5,000 rep + benzene	5,000 rep	7,500 rep	10,000 rep	alone	+ 3,000 rep	+ 5,000 rep	+ 5,000 rep
50	448	476	430	447	471	480	318	361	316	511
25	528	591	565	535	595	609	353	417	369	605
10	652	663	623	623	690	664	403	485	435	651

accelerate the development of sarcomas due to P^{32}: The mean duration to death from sarcoma is 437 days without methylcholanthrene and 318 days with it (4 tumors in each group). It does not seem possible to draw any further conclusion, even of doubtful statistical validity, from these data.

The incidences of other types of tumors, not at the site of treatment, do not show any striking differences. Lymphomas are more numerous in all groups (control and irradiated) not receiving methylcholanthrene than in those receiving it (28.8% as against 19.5%), which may be explained by the shorter survival of the latter groups. The volume of data is not sufficient for study of the age-corrected incidence. It is of interest that 3 primary tumors of heart were described grossly, on one of which microscopic examination has been made and shows a rhabdomyosarcoma.

Implanted carcinogens.—No differences in survival in the various groups were seen comparable to those in the other experiment. For reasons which are not clear, control survival was considerably greater in this experiment (table 4).

TABLE 3.—*Incidence of malignant tumors at treatment site (superficial treatment)*

	Tumors (*percent*)	$\left(\frac{\text{Total mice with tumors}}{\text{Mice alive 100 days}}\right)$	Mean duration to death (*days*)	Subcutaneous tumors (*percent*)
Controls*	1.8	(1/55)	459	0
P^{32}, 5,000 rep*	11.1	(5/45)	461	40
P^{32}, 7,500–10,000 rep	10.0	(6/60)	540	83†
MCA 1.7 mg.	16.7	(5/30)	310	0
MCA 1.7 mg. + P^{32} 3,000 rep	23.3	(7/30)	255	15
MCA 1.7 mg. + P^{32} 5,000 rep	41.4	(12/29)	303	25
MCA 0.17 mg. + 5,000 rep	13.8	(4/29)	547	25

*With and without benzene painting.
†Includes 3 malignant tumors of hibernating gland, mean duration to death 614 days.

TABLE 4.—*Days to various percentages of survival (pellet injection)*

Percent survival	Controls	Cholesterol	Stable yttrium	Y^{91}			MCA 1%	MCA 1% + Y^{91}		
				1.1 μc.	11 μc.	73 μc.	alone	+ 1.1 μc.	+ 11 μc.	+ 73 μc.
50	510	499	443	465	409	296	397	475	410	436
25	629	603	538	.....	517	479	537	557	515	547
10	715	774	632	.....	549	610	607	580	710	616

The chief difference in superficial response to the two agents was the presence of epilation over the site of the radioactive pellets, as might be anticipated on the basis of the range of the *beta* particles (over 50% of the energy will be absorbed at a distance greater than 1 mm. from the source). Similarly, carcinomas were frequent in animals irradiated by pellets (including 2 mixed tumors) and 2 of the sarcomas arose in bones close to the implantation, while only soft tissue sarcomas appeared when methylcholanthrene alone was used. Ulceration was more frequently observed in mice treated with the higher amounts of radioactivity, and reached 100 percent at the highest dose. The malignant-tumor incidences are seen in table 5.

Here we fail to see any dose-dependence of time or incidence of tumors induced by the radioactive carcinogen, possibly owing to the small numbers of animals involved. We also fail to see evidence of additivity: The mice treated with radioactive pellets containing methylcholanthrene show about the same incidence of tumors (and proportion of sarcomas) as those treated by methylcholanthrene alone. It may be suggested that the tissue subjected to chemical insult is within the area destroyed by the radiations, so that in effect the methylcholanthrene is neutralized. It is also possible that loss of pellets through ulceration may play a part. It may also be noted that, in this mode of treatment, methylcholanthrene, as well as producing no effect on life span, has no influence on pulmonary-

TABLE 5.—*Malignant tumors in treatment area (pellet injection)*

	Tumors (*percent*)	$\left(\frac{\text{Total mice with tumors}}{\text{Mice alive 100 days}}\right)$	Mean duration to death (*days*)	Carcinomas* (*percent*)
Controls	0	(0/30)	.....	.....
Cholesterol	0	(0/29)	.....	.....
Stable yttrium	0	(0/28)	.....	.....
Y^{91}, 1.1 μc	50. 0	(1/2)	473	.....
Y^{91}, 11 μc	65. 0	(13/20)	387	73
Y^{91}, 73 μc	13. 3	(2/15)	435	0
MCA 1%	40. 0	(12/30)	321	0
MCA+1.1 μc. Y^{91}	48. 1	(13/27)	433	70
MCA+11 μc. Y^{91}	34. 8	(8/23)	390	67
MCA+73 μc. Y^{91}	33. 3	(10/30)	417	57

*Of tumors diagnosed by microscopic examination.

tumor incidence, which (after the first 200 days) is 75 percent in all mice treated with methylcholanthrene, and 77 percent in those treated without it. Similarly, no effects of the various treatments on other tumors not appearing in the treated area can be made out.

Discussion

The results, as outlined above, seem to show that radioactive and chemical carcinogens are approximately additive when applied to the skin; that they are less than additive (probably due to destruction of tissue by local radiation) when applied in the form of injected pellets. They indicate that the *beta* rays used, in terms of the tissues in which tumors are induced, are more penetrating than methylcholanthrene, either applied to the skin or administered as subcutaneous pellets. It is suggested that the application of methylcholanthrene to the skin may accelerate the development of sarcoma due primarily to superficial *beta* irradiation, but this is by no means demonstrated statistically. The possibility that hydrocarbons and radioactivity might reinforce each other when each is applied in minimal amounts is not ruled out, although these experiments demonstrate that a minimal dose of methylcholanthrene has no influence on *beta*-ray carcinogenesis. Regarding papilloma formation, it does appear (table 1) that the incidence of papillomas, especially multiple papillomas, is more than additive after application of the two carcinogens; but there is no such evidence in the case of malignant tumors. It would be presuming too much to assume that this means that cancer production by the two agents is due to separate events; but the data may be taken to indicate that neither agent potentiates the other to a remarkable degree.

One striking finding is the decrease in life span following a single superficial painting with methylcholanthrene. This reduction in life expectancy cannot apparently be explained on the basis of any single pathologic state and suggests that the hydrocarbons, introduced in this way, may shorten life in a general way such as has been attributed to total-body irradiation.

It is worth mentioning, although attention has been brought to this previously (*3*, *4*), that tumor induction by hydrocarbons occurs earlier than that by exposure to radioactivity. The findings with respect to epilation and ulceration indicate an interaction between the two that is not altogether simple.

Summary

A carcinogenic hydrocarbon (methylcholanthrene) and *beta*-ray sources (P^{32} and Y^{91}) were applied, together and separately, to the skin and to the subcutaneous tissues of mice.

Carcinogenic effects of the two agents applied to skin were approximately additive, although there was a suggestion of some synergism between them in the production of papillomas.

The two agents were somewhat less than additive when given in the form of subcutaneous pellets. It is suggested that destruction of a local area of methylcholanthrene effectiveness by the high-radiation dose to this area may be responsible for this.

Application of methylcholanthrene to the skin of young adult mice in an amount of 1.7 mg. in a single painting shortens survival of mice by about one third. This effect is not shared by superficial *beta* irradiation in carcinogenic doses, by implantation of pellets containing carcinogenic doses of hydrocarbons or *beta*-emitting agents, or by the benzene solvent applied to the skin.

Literature Cited

(*1*) Cramer, W., and Stowell, R. E.: Skin carcinogenesis by a single application of 20-methylcholanthrene. Cancer Res. 3: 36–42, 1943.

(*2*) Raper, J. R., Zirkle, R. E., and Barnes, K. K.: Techniques of external irradiation with beta rays. *In* Effects of External Beta Radiation (Zirkle, R. E., ed.). National Nuclear Energy Series. New York, McGraw-Hill, 1951, pp. 1–41.

(*3*) Brues, A. M.: Carcinogenic Effects of Radiation. Advances in Biol. and Med. Res. 2: 171–191, 1951.

(*4*) Glücksmann, A.: Histogenesis of radiation-induced and of benzpyrene-induced epidermal tumors in mouse. J. Path. & Bact. 63: 176–177, 1951.

Aminoaciduria Following Total-Body Irradiation in the Human[1,2]

ELAINE J. KATZ *and* ROBERT J. HASTERLIK,[3,4]
Division of Biological and Medical Research, and Health Services Division, Argonne National Laboratory, Lemont, Ill.

Careful study of individuals accidentally exposed to varying amounts of ionizing radiation adds much to our knowledge of the mechanisms of acute radiation injury in man. Since the first military use of the atomic bomb, two accidents have occurred at the Los Alamos Scientific Laboratory as a result of temporarily uncontrolled fission reactions, causing the exposure of ten individuals to clinically significant doses of total-body radiation. The extensive clinical and laboratory studies of these individuals have been reported (*1*). Two died as a result of the exposure; one of the survivors showed the typical clinical manifestations of the acute radiation syndrome. The remaining survivors were asymptomatic, although clinical laboratory findings showed changes in the hematologic picture such as sustained lymphopenia, moderate neutropenia and thrombocytopenia, and prolonged blood-clotting time. Chemical studies of the blood did not indicate specific or marked changes. Studies of the urine chemistry, however, showed significant changes in several respects, the most striking being the excretion of large amounts of urinary amino acids and greater than normal quantities of corticosteroid-like substances. The aminoaciduria was believed to be caused by a deficiency in amino-acid utilization as well as by increased tissue breakdown, and the excretion of increased amounts of corticoid-like substances suggested adrenal dysfunction.

Experimental animal data (*2*, *3*) also have shown an increase in both the number and quantity of amino acids excreted following lethal X irradiation; with chicks (*2*) this increase was apparent one hour following the initial dose.

In a more recent accident at the Argonne National Laboratory, four laboratory workers were exposed to γ rays and a small component of neu-

[1] Received for publication November 8, 1954.
[2] Presented before the 27th Annual Meeting of the Central Society for Clinical Research, Chicago, October 30, 1954.
[3] *Present address:* Argonne Cancer Research Hospital, University of Chicago, Chicago 37, Ill.
[4] The authors would like to acknowledge the technical assistance of Miss Betty M. Van Dolah. Mathematical analysis of the data was performed by Mr. Sylvanus A. Tyler, Division of Biological and Medical Research, Argonne National Laboratory.

tron radiation at levels of 25 to 180 roentgens equivalent physical (rep). This accident gave us the opportunity to repeat some of the studies, done originally at this Laboratory, on individuals with radiation exposures not usually believed to yield clinical manifestations. Only the amino acid study will be presented here. A full clinical and laboratory report including the hematologic data will appear elsewhere (*4*).

Materials and Methods

Chromatography.—Two-dimensional chromatograms were studied, employing the original method of Dent (*5*), and using Whatman #1 chromatographic filter paper. The first solvent was phenol:water,[5] with 10 cc. of 5 percent ammonium hydroxide added to two of four bowls (200 cc. capacity) in the bottom of the case. The second solvent was collidine:-lutidine:water [6] (1:1:1.5). Solvents were changed daily and the phenol solution was freshly prepared each day. All troughs, rods, etc., were also washed daily.

Commercial "Chromatocabs" with temperature and humidity control were used; the laboratory temperature was controlled to $\pm 2°$ C. Cases were saturated with the solvent vapors for 4 to 6 hours before use. Sheets were air-dried for 24 hours in the hood at room temperature before being placed in the second solvent, and were dried in the same manner before being sprayed with ninhydrin. Color development was carried out using 0.25 percent ninhydrin in butyl alcohol with 1.0 percent acetic acid; sheets were sprayed and then allowed to dry for 24 hours in a stream of warm air at 30° C.

Reading of the sheets for identification and quantitation of amino acids was done by visual comparison of the unknown amino acids with the amino-acid standards, using a commercial 14″ × 17″ X-ray viewing box. All sheets were read exactly 24 hours after being sprayed with ninhydrin solution. Standard color tests (*5*, *6*) for the identification of questionable substances were employed. No separation was made between leucine and isoleucine; these two are considered together under the name "leucine." Amounts of amino acids measured are reported as total amino-acid mass.

Handling of urine samples:—Twenty-four-hour urine samples refrigerated during the collection period were collected from all patients and from the individuals serving as controls. Samples were collected from the four patients for the 16th-day hospital period, and subsequent samples at 4 or 5 months were obtained on three of the patients.

Controls for this specific study included two individuals followed for 7 and 8 days, respectively, and three individuals followed for 2 days each. Other control data have been obtained by the same method during the past 7 years through studies of amino-acid excretion using first-morning

[5] Mallinckrodt phenol, A. R., colorless, was used; 160 cc. boiling distilled water per pound of phenol was added to pure white phenol crystals.

[6] Colorless collidine and lutidine were obtained from Reilly Tar and Chemical Co. (Indianapolis, Ind.) and Eastman Kodak Co., respectively. The solvent-water mixture was well shaken for the first 2 days after preparation and then was allowed to remain undisturbed in the dark for 7 to 10 days. Before use, the excess water was separated from the mixture.

urine samples and 8-hour collections from approximately 350 apparently normal laboratory workers not exposed to more than the usual permissible levels of external radiation (*7*, *8*).

All analyses were done immediately; specific gravity and *p*H were determined routinely.

In order to estimate amino-acid excretion so that proper aliquots and standards could be employed, one-dimensional chromatograms were first run on each sample. Each sample was then run in triplicate, using aliquots of 25 and 50 μl., and in addition, 50 μl. of a sample concentrated 2:1 by evaporation at 100° C. In the case of both one- and two-dimensional chromatograms, the amino-acid standard solutions were run on the same sheet as the urine sample. All data are reported on the basis of the 25 μl. aliquot. The larger aliquot and the concentrated sample were employed in order to check results and to detect any abnormal amino acids that may have been present. When the identity of an unknown amino acid found in a urine sample was in question, the following two-dimensional sheets were run: 1) a sheet with a mixture of the tentatively identified amino acid added to the urine in question along with a sample of the urine alone; 2) the appropriate amino-acid standard alone along with the urine sample under investigation.

In order to obtain additional data on the handling of urine samples for large-scale amino-acid determinations, the following was done with the samples from the four patients: part of each 24-hour sample was immediately frozen; part was stored at 4° C. No preservative was added to either. The samples that were concentrated 2:1 were well stoppered and stored at 4° C. All samples stored at 4° C. were analyzed, by two-dimensional chromatograms, 2 weeks, 6 months, and 1 year after collection. All results (57 samples) were in agreement with the results obtained from the urine samples analyzed immediately after collection. The frozen samples, thawed at room temperature, were studied 6 months and 1 year after collection. The results after 6 months checked the original results. In two cases samples stored for 1 year did not yield the same results as the original samples. It would seem that bacterial and mold action do not affect the free urinary amino acids as readily as has been assumed, and that urine samples can be stored either frozen or at 4° C. without preservative for a much longer period than formerly supposed.

Clinical Data

Four individuals were accidentally exposed to γ rays and neutron radiation, and were seen within 10 minutes by members of the medical staff. At this time there were no gross clinical findings of note. Blood pressures and cardiac rates were within the normal range. Subjectively, the patients were asymptomatic except for moderate tension and apprehension. Despite the fact that all realized that they might have been exposed to quantities of radiation in the lethal range, an observation worthy of note was their quiet, self-contained behavior. The patients were hospitalized at Billings Hospital, University of Chicago Clinics.

Since at this time the magnitude of the radiation exposure was not known, all were placed at absolute bed rest. All urine and fecal samples were saved for various subsequent analyses. Complete hematologic studies, including studies of induced Na^{24} activity in the blood, were begun within 30 minutes after exposure.

Patient #1.—White male, age 31 years. Exposure 180 rep.

On admission to the hospital the patient's temperature was 37.4° C., pulse 102, respirations 20, and blood pressure 140/80.

The patient was a well developed, well nourished white male, alert and cooperative, appearing moderately apprehensive but in no other apparent distress. The physical findings were all within normal limits except for a mild erythema of the face and neck. The patient stated that he had been exposed to sunlight over the weekend and that the erythema had been present before the exposure to ionizing radiation.

Several hours after admission, the patient had one episode of nausea accompanied by vomiting. Following emesis the nausea did not reappear and the patient ate a hearty breakfast the next morning. During the remainder of his 16-day hospitalization he was asymptomatic. The erythema of the face and neck subsided and at no time subsequently has there appeared erythema of the soles of the feet, the hands or the face (the portions of the patient's body closest to the radiation source), nor has epilation occurred. The patient was discharged on the 16th day.

Following discharge from the hospital, the patient rested at home for an additional week. He returned to his work on the 23rd day after the radiation accident and has continued to feel well.

Patient #2.—White male, age 30 years. Exposure 160 rep.

On admission to the hospital the patient's temperature was 36.9° C., pulse 100, respirations 20, and blood pressure 122/70.

The patient was a well developed, well nourished white male, alert and cooperative and in no apparent distress. The physical examination revealed only findings within normal limits.

During hospitalization the patient remained completely asymptomatic and afebrile. His appetite was good at all times; there was no nausea or vomiting. None of the usual symptoms of "radiation sickness" were present in this patient. He was discharged from the hospital on the 16th day.

This patient also returned to his duties on the 23rd day after irradiation and has remained asymptomatic. Shortly thereafter he transferred his activities to another project within the Atomic Energy Commission.

Patient #3.—White female, age 49 years. Exposure 68 rep.

On admission to the hospital the patient's temperature was 36.0° C., pulse 82, respirations 20, and blood pressure 105/50.

The patient was a well developed, well nourished white female, alert and cooperative and in no apparent distress. The physical examination revealed only findings within normal limits.

On the 3rd hospital day the patient vomited on several occasions and

had four loose stools. These symptoms, however, rapidly disappeared on a bland, liquid diet and did not recur. It was the impression that this was a manifestation of anxiety and tension rather than a display of "radiation sickness." With the exception of occasional headaches, the patient remained afebrile and asymptomatic throughout her hospital stay. She was discharged from the hospital on the 16th day.

Following discharge from the hospital, the patient noted the persistence of a moderate degree of fatigue and tension. She returned to her duties 34 days after the radiation exposure and has had no symptoms attributable to late radiation effects.

Patient #4.—White male, age 29 years. Exposure: maximum of 25 rep—minimum of 8 rep.

On admission to the hospital, the patient's temperature was 36.8° C., pulse 80, respirations 20 and blood pressure 140/85.

The patient was a well developed, overnourished white male, alert and cooperative and in no apparent distress. The physical examination revealed only findings within normal limits.

The patient remained completely asymptomatic and afebrile. He had an excellent appetite and at no time noted any subjective deviation from his usual state of well being. He was discharged from the hospital on the 16th day.

The patient has continued to be asymptomatic. He returned to work on the 23rd day after the accident. On the 15th day after exposure, ophthalmoscopic examination of the fundus revealed a very small, flame-shaped hemorrhage immediately inferior to the limbus of the optic disc in the right eye. Repeat examination a month later demonstrated normal appearing fundi with no evidence of the site of the previous hemorrhage. No generalized bleeding tendency was noted and no petechiae or suggillations were observed.

Results

The values given by the five controls have been plotted in text-figure 1 to show the relationship between the concentration of amino acids excreted and the volume of urine, as well as to show the average daily excretion of amino acids. The equation

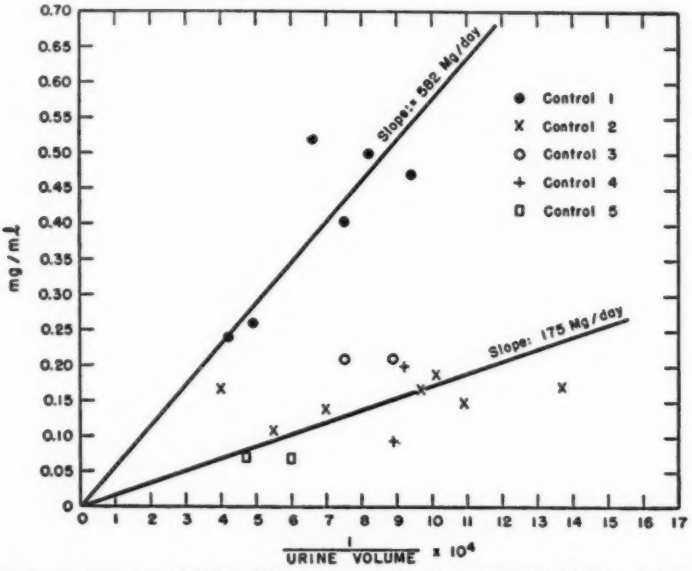
$$C\ (\text{concentration}) = \frac{T\ (\text{total excretion})}{V\ (\text{total volume})}$$

is the basis for this figure. (Or, $C = T\left(\frac{1}{V}\right)$.) If the concentration, expressed as mg. per ml., is plotted against the reciprocal of volume $\left(\frac{1}{V} \times 10^4\right)$ and a straight line passing through the point of origin results, then the slope of the resultant line is equal to the constant total amount excreted (mg./day).[7] It can be seen from the plotted data that the total amount

[7] The method of least squares is used as the fitting process with the added restriction that the line of best fit must pass through the origin of the coordinates.

excreted (mg./day) and the urine volume are independent of one another, indicating that in the normal control the total daily excretion is constant. Further analysis of the data with a plot of total daily excretion *versus* number of days of study indicates that the daily amino-acid excretion (mg./day) is independent of time (number of days) of measurement.

The total amino acids excreted by control #1 were estimated to be a constant amount (582.0 ± 73.0 mg./day).[8] The quantity of amino acids excreted by controls #2–5 was similar; the material was excreted at a rate of 175.0 ± 17.7 mg. per day.



TEXT-FIGURE 1.—Relationship between concentration and volume: control individuals.

In past work (7) with the 350 individuals previously mentioned, it has been found that most individuals excrete amino acids in a manner similar to controls #2–5. However, about 10 percent of apparently normal individuals will excrete approximately 300 to 600 mg. per day as measured by this method. The quantity of amino acids excreted per day by control #1 was decidedly higher than that of the other controls shown in text-figure 1. The values found for control #1 fall in line with the higher levels.

In the five specific controls, glycine, serine, alanine, and taurine were the major amino-acid components excreted. With the exception of control #2, glycine was excreted to the greatest extent. The number of amino acids excreted by controls #1 and #2 is shown in table 1. As in the total amounts excreted per day, the patterns of individual amino-acid excretion presented by control #1 and controls #2–5 fall in line with the general pattern previously found.

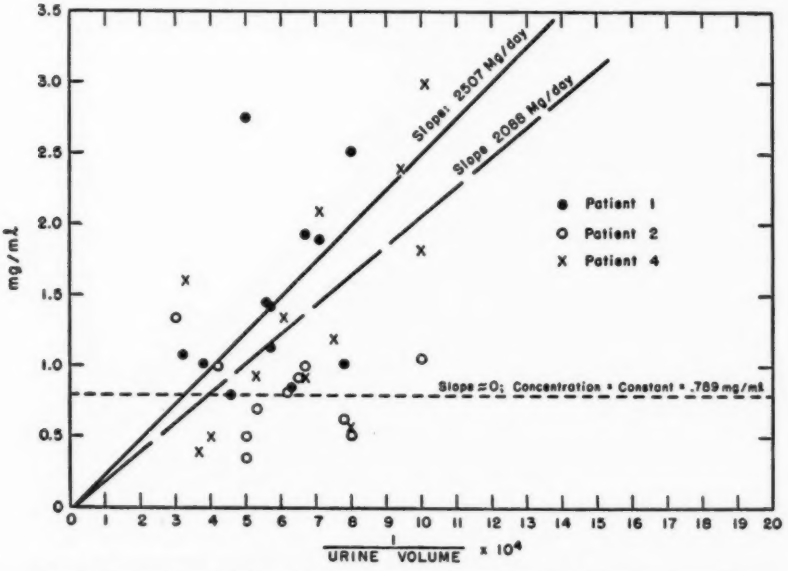
[8] Variability throughout is measured in terms of the standard error.

TABLE 1.—*Total number of individual amino acids excreted per day*

Days	12 hrs.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	*142*	*145*
Patient #1	15	...	8	7	12	...	13	11	15		10	11	10	9	9	9	12
Patient #2	12	...	9	7	10	...	10	11	11	10	9	8	7	7	4		
Patient #3	8*	...	6†	...	13	11	15	...	14	6	6	8	5	6	6	*141*/5	*155*/11
Patient #4	14	...	12	14	9	...	6	8	11	11	7	10	10	8	6	*115*/7	*130*/10
Control #1	....	7	8	4	5	5	5									*34*/7	*35*/4
Control #2	....	6	7	5	4	4	4	4									

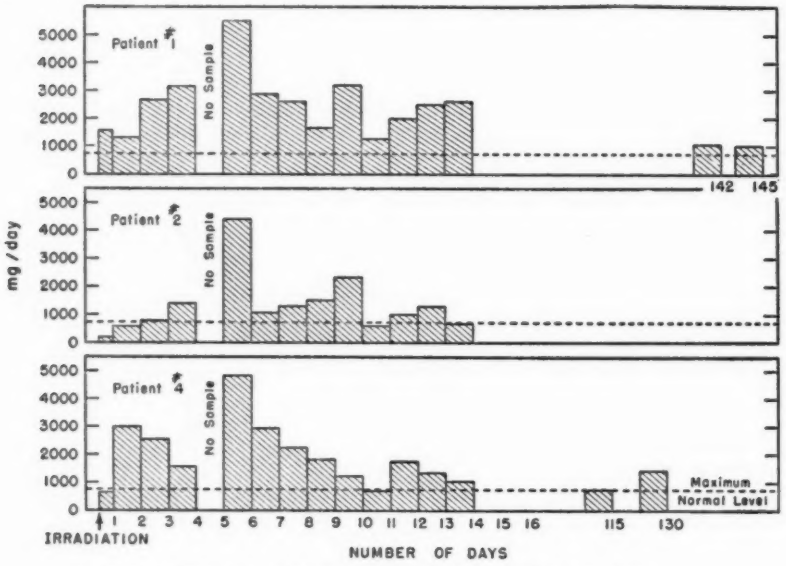
*4-hour sample. †12-hour sample.

Text-figure 2 has been plotted in the same manner as text-figure 1 to show the same excretion relationships for three of the four patients.[9] The urine samples on the day of irradiation represented a 12-hour collection period and these data are omitted. In the statistical analysis these data were given a weight of one half. Unlike the controls, there is large variance in the concentration of amino acids. Despite this variance in excretion for the patients, a line can be developed for patients #1 and #4



TEXT-FIGURE 2.—Relationship between concentration and volume: patients #1, 2, and 4.

[9] Patient #3 could not be included because of incomplete collection of her urine samples on four days.



TEXT-FIGURE 3.—Amino-acid excretion, total mg. per day.

similar to that found with the controls, which indicates that total amino-acid excretion was essentially independent of the volume of urine. If the high point of excretion (day 6) is omitted, a similar line can also be drawn for patient #2. The mathematical treatment of the data for patient #2 gives a line of slope close to zero, as shown in text-figure 2, which would indicate that total amino-acid excretion was apparently dependent on urine volume. The conclusion that this patient behaves in a manner qualitatively different from the others, with the broad implication that there are two entirely different modes of disposal of amino acids, is tenuous.

The total amino-acid excretion by patient #1 was determined mathematically from the slope of the line to be a constant amount (2,507.0 $\pm$ 317.7 mg./day). For patient #4 the excretion was 2,088.0 $\pm$ 261.1 mg. per day. The constant concentration of patient #2 is 0.789 mg. per ml.; daily excretion is 1,359.2 $\pm$ 716.3 mg. per day.

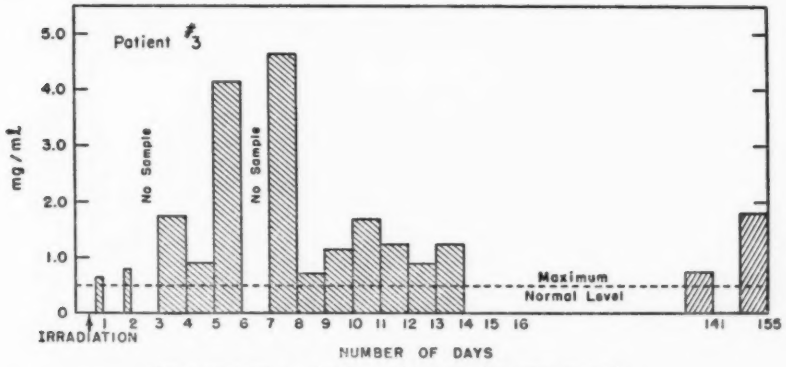
Total amino-acid excretion in mg. per day is illustrated for patients #1, #2, and #4 in text-figure 3. Although high, the value of 750 mg. per day has been chosen for our maximum normal excretion level. This is an arbitrary value derived from large numbers of control samples. During the first 12-hour period, patient #1 excreted 1,556.1 mg. of amino acids. His highest total excretion was 5,500.0 mg. during the 6th day following irradiation. Excretion greater than the maximum normal was present on analysis of the urine as late as 5 months following the irradiation exposure. Patient #2 rises above the maximum normal excretion on the 3rd day after irradiation. Patient #4 excreted 637.6 mg. during the first 12-hour period and on the second full day following irradiation excreted

2,970.0 mg. Patients #2 and #4 reached maxima, also, on the 6th day (4,422.0 mg. and 4,830.0 mg., respectively). Excretion greater than the maximum normal level was found 4 months following exposure.

All three of these patients exhibit a peak of excretion on the 6th day following irradiation. The mean of the total excretion (mg./day) was calculated along with the standard deviations of days 1 to 14, excluding day 6. In all three cases, it was found that day 6 is significantly different from this mean (with a probability of <0.05).

Because of unavoidable circumstances the collections of urine for patient #3 were incomplete on 4 days and total excretion per day could not be calculated. Therefore, excretion was expressed as concentration in mg. per ml. in the samples obtained (text-fig. 4). We have assumed a high value of 0.5 mg. per ml. as an upper limit of normal. This is also an arbitrary value derived from large numbers of control samples. Excretion on the 6th day after irradiation was 4.15 mg. per ml. Because of the incomplete collection on the 8th day, we are inclined to believe that the value of 4.67 mg. per ml. in a total 100 cc. might have been somewhat lower with a more representative sample. However, assuming that this single small sample represents a peak concentration and taking into account the data of the other patients and normal individuals, this concentration value still represents a very high excretion.

In addition to the increased quantity of amino acids there can be seen an increase in the number of individual amino acids excreted (table 1). Control individuals usually excrete measurable amounts of five or six amino acids.[10] Glycine, taurine, serine and alanine are most often found; the other amino acids are usually either valine, leucine, glutamine, or histidine. All of the patients showed a decided increase in the number found, excreting as many as 14 and 15 amino acids per day. This increase was apparent 12 hours following irradiation, and was also demonstrated as late as 5 months after the exposure.



TEXT-FIGURE 4.—Amino-acid excretion, mg. per ml.

[10] It is appropriate to emphasize that with the paper chromatographic method as here employed, using a 25 µl. aliquot, the excretion pattern is as outlined. Other amino acids are normally present in urine and may be demonstrated either by modifications of this technique or by use of other chromatographic and microbiological methods.

TABLE 2.—*Amino-acid excretion,*

	Control #1							
Day	1	2	3	4	5	6	34	35
Cysteic acid	0. 01	0. 01		0. 01		0. 04	0. 01	
Aspartic acid								
Cystine								
Glutamic acid	. 01	. 01	0. 04	. 04		. 04	. 01	
Serine	. 02	. 02	. 02	. 02	0. 02	. 04	. 02	
Glycine	. 20	. 30	. 10	. 15	. 30	. 30	. 20	0. 20
Taurine		. 04	. 075	. 04	. 05	. 10	. 02	. 01
Threonine								
Alanine	. 075	. 05			. 05		. 01	. 01
Glutamine	. 075	. 05			. 05		. 01	. 01
Tyrosine								
α-Amino-*n*-butyric	. 02							
Histidine		. 02						
Hydroxyproline								
Valine								
Lysine								
Leucine								
Arginine								
Unknown								
Total mg./ml	. 41	. 50	. 24	. 26	. 47	. 52	. 28	. 23
Urine vol. ml	1330	1220	2395	2055	1060	1490	1020	1400
Mg./day	545. 3	610. 0	574. 8	534. 3	498. 2	774. 8	275. 4	322. 0

Table 2 shows the daily amino-acid excretion of the five control individuals used for this study. With the exception of the amount of glutamic acid found in the samples of control #2, each individual presents an excretion pattern similar to that found previously in this laboratory and elsewhere (*5*, *7–12*).

Tables 3 to 6 chart the exact amino-acid excretion for all samples from the four patients. The greatest increase in excretion occurred with glycine. Other unusual increases are found with cystine, taurine, glutamic acid, aspartic acid, and hydroxyproline. The large and consistent excretion of hydroxyproline in the period under observation is particularly striking. Cysteic acid is usually considered an oxidation product of cystine, found in samples that have been stored for several days. Since there was no storage in these cases, cysteic acid can be considered a valid

control individuals, mg./ml.

Control #2							Control #3		Control #4		Control #5	
1	2	3	4	5	6	7	1	2	1	2	1	2
0. 05	0. 05	0. 05	0. 05	0. 05	0. 05	0. 05				0. 01	0. 01	0. 01
. 02	. 02	. 02	. 02	. 02	. 02	. 02			0. 025	. 025		
. 02	. 02	. 05	. 02	. 02	. 05	. 05	0. 20	0. 20	. 05	. 05	. 05	. 05
. 05	. 02	. 05	. 02	. 05	. 05	. 05			. 01	. 01	. 01	. 01
	. 01						. 01	. 01	. 01	. 01		
. 01	. 01						. 01	. 01	. 01	. 02		
. 02	. 02	. 02										
										. 025		
										. 025		
										. 025		
. 17	. 15	. 19	. 11	. 14	. 17	. 17	. 21	. 21	. 095	. 200	. 07	. 07
730	920	990	1830	1430	1030	2500	1335	1120	1120	1080	2130	1670
124. 1	138. 0	188. 1	201. 3	200. 2	175. 1	425. 0	280. 4	235. 2	106. 4	216. 0	149. 1	116. 9

constituent. Small amounts of cysteic acid were also found in the urine of control #1.

Using control #1 as an example of maximum control excretion and the other four controls as examples of average control excretion, the extreme aminoaciduria of these patients is evident. Measured with this method, controls almost always show a maximum excretion of glycine, with lesser amounts ($<$0.05 mg./ml.) of other amino acids. Cystine, cysteic acid, and aspartic acid are rarely found in the urine of control individuals; glutamic acid appears fairly often in small amounts.[11] Although hydroxy-

[11] This has been reported as a normal constituent by some authors; others claim that it does not appear in appreciable amounts in freshly voided urine (*12*). Although these samples were run immediately after collection and were refrigerated for the collection period, they cannot be considered as freshly voided urine samples. Other samples run in this laboratory have always been refrigerated for about 4 hours before analysis.

TABLE 3.—*Amino-acid excretion, patient #1, mg./ml., postirradiation*

Day	12 hrs.	2	3	4	5*	6	7	8	9	10	11	12	13	14	142	155
Cysteic acid	0. 10	0. 02	0. 05	0. 05		0. 05	0. 02	0. 02	0. 05	0. 05	0. 05	0. 02	0. 05	0. 05	0. 02	0. 02
Aspartic acid	. 05			. 20		. 02	. 05	. 05		. 02	. 02	. 02				. 02
Cystine	. 20	. 02					. 05	. 05						. 20		
Glutamic acid	. 10	. 05	. 10	. 10		. 10	. 10	. 05	. 05	. 02	. 05	. 075	. 10	. 10	. 02	. 02
Serine	. 40			. 40				. 10		. 05					. 05	. 05
Glycine	. 50	. 40	. 25	. 50		1. 00	. 75	. 75	. 20	. 50	. 20	. 50	. 50	. 50	. 05	. 10
Taurine	. 25	. 10	. 05	. 10		. 20	. 20	. 10	. 10	. 05	. 05	. 10	. 10	. 20		. 02
Threonine	. 05			. 10		. 10		. 05								. 02
Alanine	. 05	. 05	. 05	. 10		. 10	. 10	. 10	. 02	. 01	. 01	. 02	. 05	. 05	. 01	. 02
Glutamine	. 20			. 25		. 20		. 20	. 02	. 02	. 02				. 02	. 05
Tyrosine																
α-Amino-*n*-butyric						. 05										
Histidine	. 40	. 10		. 20		. 10	. 10	. 10	. 10	. 10	. 10	. 10	. 10	. 10	. 10	. 10
Hydroxyproline	1. 00	. 10	. 50	. 50		. 75	. 50	. 25	. 25	. 25	. 50	. 25	. 50	. 25	. 10	. 10
Valine	. 02		. 01	. 02		. 01	. 01	. 02			. 01	. 01	. 01	. 01	. 01	. 01
Lysine	. 05					. 05	. 05	. 05				. 02	. 02			
Leucine	. 05							. 01								
Arginine																
Unknown						. 02										
Total mg./ml	3. 42	. 84	1. 01	2. 52		2. 75	1. 93	1. 90	. 79	1. 07	1. 01	1. 12	1. 43	1. 46	. 38	. 53
Urine vol. ml	455	1585	†2525	1255		2000	1500	1400	2150	3000	1275	‡1900	§1600	1800	2890	1950
mg./day	1556. 1	1331. 4	2661. 1	3162. 6		5500. 0	2895. 0	2660. 0	1698. 5	3210. 0	1287. 8	1999. 4	2496. 0	2628. 0	1098. 2	1033. 5

*No sample obtained. †23-hour sample. ‡26-hour sample. §22-hour sample.

TABLE 4.—*Amino-acid excretion, patient #2, mg./ml., postirradiation*

Day	12 hrs.	2	3	4	5*	6	7	8	9	10	11	12	13	14
Cysteic acid	0. 10	0. 02		0. 05		0. 05	0. 05	0. 05	0. 02	0. 05	0. 02	0. 02	0. 02	0. 02
Aspartic acid	. 05			. 10		. 02	. 05	. 05	. 02				. 05	
Cystine	. 05	. 05		. 10		. 10	. 02	. 05	. 05	. 05	. 05			
Glutamic acid	. 10	. 10	0. 05	. 05		. 05	. 10	. 05	. 05	. 05	. 02	. 05	. 05	. 02
Serine	. 10													
Glycine	. 15	. 20	. 20	. 20		. 50	. 50	. 20	. 20	. 50	. 20	. 20	. 50	. 30
Taurine		. 05	. 05			. 05	. 05	. 05					. 10	
Tyreonine														
Alanine	. 10	. 02	. 01	. 01		. 01	. 02	. 01	. 05	. 01	. 01	. 02	. 01	. 01
Glutamine	. 10							. 02		. 02	. 01	. 01		
Tyrosine														
α-Amino-*n*-butyric														
Histidine	. 10	. 10	. 05	. 10		. 05	. 10	. 10	. 10	. 05	. 10			
Hydroxyproline	. 25	. 10	. 25	. 25		. 50	. 10	. 10	. 50	. 25	. 10	. 10	. 10	
Valine	. 02	. 02	. 01	. 01		. 01	. 01	. 01		. 01				
Lysine	. 10			. 05			. 05		. 01			. 10		
Leucine									. 01					
Arginine														
Unknown														
Total mg./ml	1. 22	. 66	. 62	. 92		1. 34	1. 05	. 69	1. 01	. 99	. 51	. 50	. 83	. 35
Urine vol. ml	155	900	†1225	1540		3300	1000	1900	1500	2400	1250	2000	1600	2000
mg./day	189. 1	594. 0	792. 5	1416. 8		4422. 0	1050. 0	1311. 0	1515. 0	2376. 0	637. 5	1000. 0	1328. 0	700. 0

*No sample obtained. †23-hour sample.

TABLE 5.—*Amino-acid excretion, patient #3, mg./ml., postirradiation*

Day	4 hrs.	12 hrs.	3*	4	5	6	7*	8	9	10	11	12	13	14	141	155
Cysteic acid	0. 10			0. 10	0. 05	0. 10		0. 05								0. 05
Aspartic acid	. 05	0. 05		. 05	. 05	. 10		. 02					0. 05	0. 10		. 05
Cystine				. 10	. 05	. 05		. 05								. 05
Glutamic acid		. 05		. 20	. 20	1. 00		. 50	0. 05	0. 075	0. 10	0. 10	. 10	. 10	0. 05	. 10
Serine																
Glycine	. 20	. 40		. 75	. 05	2. 00		1. 75	. 50	. 75	1. 00	. 50	. 50	. 75	. 40	. 75
Taurine	. 05	. 10		. 10	. 10	. 20		. 25	. 02	. 02	. 20	. 10	. 10	. 10		. 10
Threonine						. 05		. 10								
Alanine	. 02	. 05		. 10	. 20	. 10		. 50	. 01	. 01	. 05	. 05	. 05	. 10	. 02	. 05
Glutamine				. 01		. 05		. 50							. 02	
Tyrosine																
α-Amino-*n*-butyric				. 02	. 01	. 05		. 50								
Histidine	. 10	. 05		. 10	. 10	. 10		. 20			. 05			. 10		. 10
Hydroxyproline	. 10	. 10		. 10	. 10	. 10		. 10	. 10	. 25	. 25	. 50	. 10		. 25	. 50
Valine	. 01			. 01	. 01	. 10		. 05			. 01					. 01
Lysine				. 10		. 05		. 10								. 05
Leucine						. 10										
Arginine									. 05	. 05	. 05					
Unknown																
Total mg./ml	. 63	. 80		1. 74	. 92	4. 15		4. 67	. 73	1. 16	1. 71	1. 25	. 90	1. 25	. 74	1. 81
Urine vol. ml	60	545		†460	1000	†500		†100	900	†500	850	750	1100	1500	1500	1500
mg./day	37. 8	436. 0			920. 0				657. 0		1453. 5	937. 5	990. 0	1875. 0	1110. 0	2640. 0

* No sample obtained.
† Incomplete sample.

† Incomplete sample.

TABLE 6.—*Amino-acid excretion, patient #4, mg./ml., postirradiation*

Day	12 hrs.	2	3	4	5*	6	7	8	9	10	11	12	13	14	115	130
Cysteic acid	0. 10	0. 10	0. 10	0. 05				0. 05	0. 10	0. 05	0. 05	0. 02	0. 02	0. 02	0. 02	0. 05
Aspartic acid	. 15						0. 10	. 05	. 02	. 05	. 02	. 05	. 05	. 05	. 02	. 05
Cystine	. 20	. 05	. 10				. 05	. 05	. 10							
Glutamic acid	. 20	. 20	. 20	. 05		0. 20	. 50	. 10	. 20	. 05	. 10	. 05	. 05	. 05	. 02	. 02
Serine	. 10		. 20													. 10
Glycine	1. 50	1. 00	. 50	. 50		1. 00	. 50	. 50	. 75	. 10	. 10	. 50	. 50	. 20	. 20	. 40
Taurine	. 02	. 05	. 10	. 02				. 05			. 02	. 05	. 05	. 05		
Threonine																
Alanine	. 10	. 20	. 20	. 05		. 05	. 10	. 10	. 10	. 05	. 05	. 01	. 10	. 02	. 05	. 05
Glutamine	. 20		. 20	. 05				. 05	. 05		. 02					. 10
Tyrosine																
α-amino-*n*-butyric			. 05													
Histidine	. 75	. 40	. 20	. 20		. 10	. 10	. 10	. 20	. 10	. 10	. 10	. 10		. 10	. 10
Hydroxyproline	1. 00	. 75	. 25	. 25		. 25	. 75	. 25	. 25	. 10	. 10	. 10				. 05
Valine	. 30	. 05	. 10			. 01	. 01	. 05	. 01		. 01	. 01				. 02
Lysine		. 10	. 10						. 05			. 05	. 05		. 02	
Leucine	. 30	. 05														
Arginine	. 10	. 05	. 10	. 02												
Unknown																
Total mg./ml	5. 02	3. 00	2. 40	1. 19		1. 61	2. 11	1. 35	1. 83	. 50	. 57	. 94	. 92	. 39	. 43	. 94
Urine vol. ml	127	990	†1015	1340		3000	1400	1650	1000	2500	1250	1900	1500	2700	1750	1535
mg./day	637. 6	2970. 0	2541. 9	1594. 6		4830. 0	2954. 0	2227. 5	1830. 0	1250. 0	712. 5	1786. 0	1380. 0	1053. 0	752. 5	1442. 9

* No sample obtained.
† 23-hour sample.

proline has been found in samples from apparently normal individuals (*5*), it has never been found in any control sample run by this method in this laboratory. It has previously been found in other severely irradiated patients (*8*).

Amino-acid excretion in the four patients, as in the controls, was calculated on the basis of the percentage of the total excretion. No pattern of any significance could be discerned either in the individual patients or in the group as a whole.

The specific gravity and *p*H of all samples were routinely determined. No samples gave abnormal or unusual results.

The volume of the urine excreted varied between 750 and 3,300 ml. per day, and seemed to be the normal urinary variation.

Discussion

Investigation of the amino acids of normal urine by microbiological, chemical, and paper chromatographic means has been extensive. Differences in technique and in the sensitivity of methods have caused great variance in results, so that it is often difficult to compare data obtained by dissimilar methods. A recent paper (*12*) presents quantitative values for amino acids in the urine of young adult males (data obtained by a chromatographic technique employing columns of Dowex 50 ion-exchange resin) and presents a good discussion of values obtained by several methods. A comparison of findings in normal urines analyzed by various techniques (*9–20*) with results obtained by us and others using the paper-chromatographic method show that there is general agreement between the three methods (microbiological, chemical, and paper chromatographic). Results obtained by the microbiological method, however, tend to be somewhat higher than those obtained by other methods. As pointed out by Stein (*12*), comparison with the microbiological method is difficult because amino acids exist in urine in combined forms which have an unknown microbiological availability.

In general, the results obtained in this laboratory by the paper-chromatographic method agree well with those obtained by Stein using the quantitative ion-exchange method. Discrepancy in total amounts can be ascribed to differences in sensitivity of methods and to the fact that Stein has identified, as far as possible, all of the ninhydrin-reacting constituents of normal urine. Most workers using paper chromatography cite a far smaller amount of histidine than is found by workers using other methods. This difference is undoubtedly caused by difficulty in estimating histidine on paper chromatograms because of its extremely weak ninhydrin reaction. This does not obtain to an appreciable extent with other amino acids.

As has been mentioned, using this paper-chromatographic method, the urines of control individuals show five or six amino acids: glycine, taurine, serine, alanine, and one or more from among valine, leucine, glutamine, and histidine. Other amino acids such as α-amino-*n*-butyric acid, tyrosine, threonine, methylhistidine, lysine, and arginine are occasionally found. No urine from normal individuals has ever shown methionine or

phenylalanine. Cystine, cysteic acid, and aspartic acid are rarely found; glutamic acid appears fairly often in small amounts. Stein (*12*) states that the glutamic-acid content of normal urine increases on standing, even at 4° C. All individual samples run in this laboratory have been analyzed between 2 and 4 hours after collection, or after the close of a 24-hour collection. Such samples cannot be considered as freshly voided. In the samples that had been stored at 4° C., however, there was no change in glutamic-acid content.

The criterion for the selection of average control values has been discussed, and maximum levels of excretion in the normal control in mg. per ml. and mg. per day were selected. The greatly elevated excretion of urinary amino acids by all four patients is evident and clear-cut. This increase over average normal is similar to that found with lethal and near-lethal levels of irradiation (*8*). It is an increase greater than that reported in the literature for a variety of disease states in which marked aminoaciduria is a feature (*5, 9–11, 14, 17, 21–33*), or in which marked aminoaciduria was produced as a result of ACTH or cortisone therapy (*20, 34–36*). Only in some cases of cystinuria (*23*), Fanconi syndrome (*10*), chronic cirrhosis with necrosis (*17*), acute yellow atrophy (*10, 29*), and severe chemical and thermal burns (*25, 31*) did levels approach those found in the irradiated patients.

In the severely irradiated patients of the Los Alamos series the peak of clinical symptoms occurred on the 6th day following the accident; on this day the greatest amino-acid excretion was observed. In the present series this phenomenon of peak aminoaciduria is also evident although the patients were asymptomatic. It would seem that the mechanism or one of the mechanisms operative in the production of aminoaciduria following X irradiation reaches a peak on the 6th day after exposure without obvious relation to clinical condition.

The less severely irradiated workers involved in the Los Alamos accidents with exposures somewhat comparable to those in the present accident[12] showed some discrepancy in the blood picture but did not exhibit the marked aminoaciduria characteristic of this group of four patients. In the former accident there was abnormal excretion evident in a urine sample from one patient collected 6 hours after the accident and, in addition, another patient showed abnormal excretion the day following the accident. These results at the lowest level are at variance with our findings. The reason is not apparent at present.

The response is not proportional to dosage. Although patient #1 excreted a greater total amount of amino acids and a somewhat larger number of individual amino acids than the other three patients, the difference did not correspond to the difference in dosage. Patient #4 with an exposure in the range of 8 to 25 rep exhibited results similar to those shown by patient #1, and all four patients in turn gave results similar to those exhibited by the surviving Los Alamos worker hospitalized with acute

[12] Exact dose computation was not possible (*1*).

radiation sickness. Total amino-acid excretion in the two fatal cases was, on the other hand, considerably higher than that reported here.

The response at 12 hours is also worthy of note, indicating, certainly, some change starting at an even earlier time. In the case of the Los Alamos patient with near-lethal exposure, marked increase was found in a urine sample obtained 6 hours after exposure.

Hematologic studies of the present accident cases show that, although the rate and magnitude of the drop in absolute lymphocyte and total white count are roughly proportional to the radiation exposure level (*4*), aminoaciduria seems to be a significantly more sensitive and striking early indicator of radiation exposure.

Bone-marrow studies on all four individuals done on the 10th day following exposure demonstrated no abnormalities. The depth of total white-cell depression was not reached until approximately 1 month after irradiation, though in the lymphocyte count the trend toward decrease was apparent on the first day in the patient with the highest exposure. Total cell-count changes in the patients with the two lowest exposure levels were not a sensitive indicator of the exposure. Cytologic changes similar to those observed and described previously (*1*) were present in the lymphocytes, and to a lesser extent in the neutrophils. These changes were also evident in the white cells of the peripheral blood of patient #4 who had the lowest level of radiation.

Further evidence that the aminoaciduria is not a quantitative response comes from a comparison with the disease states previously mentioned, in which the aminoaciduria of Wilson's disease (*14*, *27*, *28*), diabetic ketosis (*24*), progressive muscular dystrophy (*26*), acute cirrhosis (*17*), subacute cirrhosis with necrosis (*17*), and lead poisoning (*32*), for example, does not reach the levels found in the irradiated patients.

The apparently nonspecific excretion of amino acids also seems indicative of a nonquantitive response. Glycine, hydroxyproline, cystine, taurine, and glutamic acid increased to the greatest extent over amounts normally excreted. In one of the lethal cases there was a massive excretion of taurine (up to 3.0 mg./ml.), but excretion of hydroxyproline similar to that found here. Large excretion of taurine in cases of severe thermal burns (*31*) is in line with a knowledge of generalized tissue breakdown. The two lethal cases demonstrated marked tissue breakdown from radiation burns and gangrene, a condition not found in our patients.

It seems that there is a general increase in the number and quantity of individual amino acids in the urine, but that this response is not quantitatively related to radiation dosage. Apparently in the four patients studied by us extremely radiosensitive metabolic factors are operative, since the response is present during the first 12 hours and at low radiation levels. The observation that the aminoaciduria reached a peak at 6 days in all patients suggests that consistent and similar metabolic changes are operative in all.

Studies of the acute radiation syndrome have shown a widespread tissue destruction throughout the body. Secondary reactions following radia-

tion exposure, such as infection, fluid loss, and presumably the adrenal response, are the same as those found in syndromes in which delayed tissue death is a prominent feature, as in thermal burns, intestinal obstruction, and massive gangrene (*1*). According to this thesis, it was thought that the increased protein degradation in dead and dying cells was a major cause of the aminoaciduria observed in the heavily irradiated workers (*8*). This was consistent with the correspondence of amino-acid excretion to the clinical course. In view of the magnitude of the aminoaciduria and the early response at such low dose levels, it is difficult for us to believe that increased protein breakdown is the major factor in producing the aminoaciduria found in our patients, although this may be an important factor in heavily irradiated individuals.

Aminoaciduria occurring after irradiation may be related to the following: 1) decreased oxidation and utilization of amino acids by the liver and other tissues; 2) partial inhibition of amino-acid metabolism in the kidney and partial inhibition of enzyme systems concerned with renal tubular resorption of amino acids; 3) adrenal response to a stress situation.

Liver disease or damage as usually described does not give rise to aminoaciduria of the extent found here (*11*, *13*, *21*). Such diseases have recently been studied and classified according to amino-acid excretion (*11*). In mild liver disease only cystinuria is found. As the degree of liver disease increases, there is an increase in the number of amino acids excreted without a marked increase in the quantity of each excreted, and this progresses to the condition in which massive aminoaciduria is manifest, as in acute yellow atrophy or serious jaundice. The pattern given by our four patients fits the pattern given by patients severely ill with certain types of liver disease. Since there is no massive impairment of other liver functions or extensive tissue destruction, it would seem that decreased oxidation and utilization of the amino acids by the liver and other tissues may be an explanation for the aminoaciduria. Such an explanation seems to fit the response given by postoperative patients with no kidney damage (*21*, *31*), for in such cases there is urinary amino-acid excretion similar to that given by patients with diagnosed liver damage. Using liver biopsy (*21*), cytologic evidence of damage to the hepatic cells may be seen a few hours after surgery without other evidence of functional change, and may be related in general to a "stress reaction."

On the other hand, there is no defect in the ability of the liver of the irradiated animal to deaminate amino acids or to incorporate them into protein (*8*, *37*).

In an extensive study of aminoaciduria in various types of liver disease and dysfunction, Dent (*21*) assumes that a rise in urinary output of amino acids reflects a raised plasma level, with the kidney playing no primary part in the production of the aminoaciduria. Since plasma amino-acid levels were not measured on our patients, it is not possible to evaluate this aspect.

Specific necrosis of the renal tubules is not the cause of the aminoaciduria, as is the case with the aminoaciduria of heavy metal poisoning.

Other studies of aminoaciduria in liver disease showed altered urinary amino-acid levels in patients in whom the factor of renal damage had been excluded by the usual clinical and laboratory tests of renal function (*17, 31*).

It has been postulated that competitive tubular resorption may be a factor in the specific aminoaciduria that occurs when the renal system is overloaded with one or more amino acids (*13, 38*). This has been proposed, for example, as one possible cause for the excessive excretion of seven amino acids in pregnancy (*13, 18*). In the aminoaciduria of muscular dystrophy competition for resorption may be offered by creatinine (*13*). Other data (*39*) show great variation in relative renal clearance of various amino acids, suggesting that the renal tubules may possess marked selectivity in their normal resorptive function.

Some interference in the amino-acid-oxidative mechanism in the kidney and other tissues seems a more logical assumption in explaining the gross aminoaciduria of the patients receiving low levels of radiation. A partial inhibition of amino-acid oxidation in the kidney of animals injured by ionizing radiation at 100 r has been shown (*40*). Studies of cystinuria have led to the postulation of an enzymatic defect in the kidney, a defect which affects the resorption of certain amino acids (arginine, lysine, the sulfur amino acids, and taurine) (*11, 23*), for feeding studies show that there is no defect in the metabolism of these amino acids (*11*). An enzymatic defect causing defective resorption has also been postulated as a factor in the gross aminoaciduria of Fanconi syndrome (*10*). It is interesting to note that in Wilson's disease (hepatolenticular degeneration) gross aminoaciduria may occur with only minimal, if any, evidence of liver dysfunction (*14*) and with no clinical or laboratory evidence of renal disease (*27*). In Wilson's disease there is abnormal excretion of all the common amino acids along with cystine, lysine, and arginine. The last three amino acids are major excretion components of cystinuria.

The administration of ACTH and cortisone has been followed by extensive aminoaciduria (*20, 34–36*). This has been explained partly as a consequence of damage to the renal tubules (*42*). Other explanations postulate an inhibition of protein synthesis (*43, 44*) and accelerated mobilization of protein (*45*).

In the Los Alamos accident an interesting finding was increased adrenocorticoid excretion, similar to that found with an acute thermal burn (*1*). Some animals have shown a similar response (*41*). The adrenal response has been given as a partial explanation of the aminoaciduria found in a group of patients with severe burns (*31*). In the latter group the aminoaciduria was proportional to the severity of the trauma. The aminoaciduria in diabetic ketosis has likewise been postulated as having been caused by increased secretion of adrenocorticotropic hormone (*24*). A comparison of the free amino acids of the tissues of normal and cortisone-injected chick embryos showed that the content of free hydroxyproline increased markedly in the tissues of cortisone-treated embryos (*46*). In some in-

stances there was an increase in glycine; there was no consistent change or increase in other free amino acids.

None of our patients showed gross evidence of tissue destruction from burns or gangrene. It is evident cytologically that changes had occurred in the internal metabolism of the lymphocyte and other white blood cells or their precursors. It is conceivable that even at the lower dose levels changes occurred in several physiologic mechanisms concerned with the total metabolism of amino acids. These include the deamination, transamination, and oxidation systems, and the mechanisms concerned with the resorption of amino acids by the renal tubules. It is not believed that gross protein breakdown associated with tissue necrosis was a significant factor.

Summary

1) Following the accidental exposure of four laboratory workers to total-body irradiation with γ rays and neutrons at levels between 25 and 180 rep (roentgens equivalent physical), total daily urinary amino-acid excretion increased to as high as ten times normal values. The quantities of individual amino acids excreted varied from two to twenty times normal amounts. The most marked excretion occurred with hydroxyproline and glycine.

2) Abnormal excretion in both number and quantity of amino acids occurred as early as 12 hours following the initial exposure, indicating that the physiologic changes had become operative before this time. Abnormal amino-acid levels were evident 5 months following exposure.

3) Aminoaciduria is a sensitive and early indicator of low levels of radiation exposure.

4) There was no direct quantitative relationship between the radiation level and the degree of aminoaciduria.

5) It is believed that the aminoaciduria occurring after irradiation might result from the following: 1) decreased oxidation and utilization of amino acids by the liver and other tissues; 2) adrenal response to a stress situation; 3) changes in the threshold of renal tubular resorption of amino acids; 4) increased protein (tissue) breakdown. In view of the response to an exposure of 25 rep, it is postulated that increased protein breakdown is not a major factor in producing the aminoaciduria found in these patients.

Bibliography

(*1*) Hempelmann, L. H., Lisco, H., and Hoffman, J. G.: The acute radiation syndrome: A study of nine cases and a review of the problem. Ann. Int. Med. Part I. 36: 279–510, 1952.

(*2*) Katz, E. J., and Stearner, S. P.: Early biochemical changes in the plasma of X-irradiated chicks. To be published.

(*3*) Kay, R. E., and Entenman, C.: Free amino acids in the tissues and urine of the X-irradiated rat. (Abstract.) Fed. Proc. 13: 520, 1954.

(*4*) Hasterlik, R. J., Jacobson, L. O., Hathaway, E. A., and Skirmont, E.: In preparation.

(5) DENT, C. E.: A study of the behavior of some sixty amino-acids and other ninhydrin-reacting substances on phenol-"collidine" filter paper chromatograms, with notes as to the occurrence of some of them in biological fluids. Biochem. J. 43: 169–180, 1948.

(6) BLOCK, R. J.: Paper Chromatography. A Laboratory Manual. New York, Academic Press, 1952.

(7) KATZ, E. J.: Unpublished data.

(8) KATZ, E. J.: *In* Hempelmann, L. H., *et al.*: *Op. cit.* (1), sect. 7, pp. 428–445.

(9) DENT, C. E.: Detection of amino-acids in urine and other fluids. Lancet 251: 637–639, 1946.

(10) ———.: The aminoaciduria in Fanconi syndrome. Biochem. J. 41: 240–253, 1947.

(11) ———.: Quelques désordres du métabolisme des acides aminés. Exposés ann. biochim. méd. 13: 79–97, 1951.

(12) STEIN, W. H.: A chromatographic investigation of the amino acid constituents of normal urine. J. Biol. Chem. 201: 45–58, 1953.

(13) BRICK, I. B.: Medical progress; the clinical significance of aminoaciduria. New England J. Med. 247: 635–644, 1952.

(14) COOPER, A. M., ECKHARDT, R. D., FALOON, W. W., and DAVIDSON, C. S.: Investigation of the aminoaciduria in Wilson's disease (hepatolenticular degeneration): Demonstration of a defect in renal function. J. Clin. Invest. 29: 265–278, 1950.

(15) ECKHARDT, R. D., COOPER, A. M., FALOON, W. W., and DAVIDSON, C. S.: Urinary excretion of amino acids in man. Tr. New York Acad. Sc. 10: 284–290, 1948.

(16) HIER, S. W.: Urinary excretion of individual amino acids on normal and low protein diets. Tr. New York Acad. Sc. 10: 280–283, 1948.

(17) DUNN, M. S., AKAWAIE, S., YEH, H. L., and MARTIN, H. F.: Urinary excretion of amino acids in liver disease. J. Clin. Investigation 29: 302–312, 1950.

(18) WALLRAFF, E. B., BRODIE, E. C., and BORDEN, A. L.: Urinary excretion of amino acids in pregnancy. J. Clin. Investigation 29: 1542–1544, 1950.

(19) HARPER, H. A., HUTCHIN, M. E., and KIMMEL, J. R.: Concentrations of nineteen amino acids in plasma and urine of fasting normal males. Proc. Soc. Exper. Biol. & Med. 80: 768–771, 1952.

(20) BORDEN, A. L., BRODIE, E. C., WALLRAFF, E. B., HOLBROOK, W. P., HILL, D. F., STEPHENS, C. A. L., JOHNSON, R. B., and KEMMERER, A. R.: Amino acid studies and clinical findings in normal adults and rheumatoid arthritis patients treated with ACTH. J. Clin. Invest. 31: 375–379, 1952.

(21) DENT, C. E., and WALSHE, J. M.: Amino acid metabolism in liver disease. *In* Liver Disease. Philadelphia, Blakiston, 1951, pp. 22–32.

(22) YEH, H. L., FRANKL, W., DUNN, M. S., PARKER, P., HUGHES, B., and GYÖRGY, P.: Urinary excretion of amino acids by a cystinuric subject. Am. J. M. Sc. 214: 507–512, 1947.

(23) STEIN, W. H.: Excretion of amino acids in cystinuria. Proc. Soc. Exper. Biol & Med. 78: 705–708, 1951.

(24) GRAY, C. H., and ILLING, E. K. B.: Plasma and urinary amino acids in diabetes. J. Endocrinol. 8: 44–49, 1952.

(25) SPENCER, A. G., and FRANGLEN, G. T.: Gross aminoaciduria following lysol burn. Lancet 262: 190–193, 1952.

(26) AMES, S. R., and RISLEY, H. A.: Aminoaciduria in progressive muscular dystrophy. Proc. Soc. Exper. Biol. & Med. 68: 131–135, 1948.

(27) SPILLANE, J. D.: Aminoaciduria. Lancet 257: 915, 1949.

(28) UZMAN, L., and DENNY-BROWN, D.: Amino-aciduria in hepatolenticular degeneration (Wilson's disease). Am. J. M. Sc. 215: 599–611, 1948.

(29) LAWRIE, N. R.: The excretion of l(—)-tyrosine in urine. Biochem. J. 41: 41–44, 1947.

(31) NARDI, G. L.: "Essential" and "nonessential" amino acids in the urine of severely burned patients. J. Clin. Investigation 33: 847–854, 1954.

(32) WILSON, V. K., THOMSON, M. L., and DENT, C. E.: Aminoaciduria in lead poisoning. A case in childhood. Lancet 265: 66–68, 1953.

(33) ROTHSTEIN, A., and BERKE, H.: Aminoaciduria in uranium poisoning. I. Use of amino acid nitrogen to creatinine ratio in "spot" samples of urine. J. Pharmacol. & Exper. Therap. 96: 179–187, 1949.

(34) BRODIE, E. C., WALLRAFF, E. B., BORDEN, A. L., HOLBROOK, W. P., STEPHENS, C. A. L., JR., HILL, D. F., KENT, L. J., and KEMMERER, A. R.: Urinary excretion of certain amino acids during ACTH and cortisone treatment of rheumatoid arthritis. Proc. Soc. Exper. Biol. & Med. 75: 285–287, 1950.

(35) RONZONI, E., ROBERTS, E., FRANKEL, S., and RAMASARMA, G. B.: Influence of administration of ACTH on urinary amino acids. Proc. Soc. Exper. Biol. & Med. 82: 496–503, 1953.

(36) BERGENSTAL, D. M., LANDAU, R. L., KIRSNER, J., and LUGIBIHL, D.: Effects of ACTH and related hormones on amino acid metabolism. Proc. Second Clinical ACTH Conf. *In* Research (Mote, J. R., ed.). Vol. 1. New York, Blakiston, 1951, pp. 250–257.

(37) ABRAMS, R.: Effect of X-rays on nucleic acid and protein synthesis. Arch. Biochem. 30: 90–99, 1951.

(38) RIGGS, T. R., COYNE, B. A., and CHRISTENSEN, H. N.: Concentrative uptake of ultraviolet-absorbing amino acids by cells. (Abstract.) Fed. Proc. 12: 258–259, 1953.

(39) SHEFFNER, A. L., KIRSNER, J. B., and PALMER, W. L.: Studies on amino acid excretion in man. I. Amino acids in urine. J. Biol. Chem. 175: 107–115, 1948.

(40) BARRON, E. S. G.: Effects of X-rays on tissue metabolism. Atomic Energy Commission Document, AECD–2316. Washington, U. S. Gov't Printing Office, 1946.

(41) PATT, H. M., and BRUES, A. M.: The pathological physiology of radiation injury in the mammal. II. Specific aspects of the physiology of radiation injury. *In* Radiation Biology, Vol. I. High Energy Radiation (Hollaender, A., ed.). New York, McGraw-Hill, 1954, pp. 959–1028.

(42) MOTE, J. R., ed.: Proc. Second Clinical ACTH Conf. *In* Research. Vol. I., sect. 2. New York, Blakiston, 1951.

(43) CLARK, I.: The effect of cortisone upon protein synthesis. J. Biol. Chem. 200: 69–76, 1953.

(44) LAWRENCE, A. W., and MACVICAR, R.: Effect of cortisone on protein synthesis in the rat. (Abstract.) Fed. Proc. 13: 248, 1954.

(45) ROBERTS, S.: The influence of the adrenal cortex on the mobilization of tissue protein. J. Biol. Chem. 200: 77–88, 1953.

(46) ROBERTS, E., KARNOFSKY, D. A., and FRANKEL, S.: Influence of cortisone on free hydroxyproline in the developing chick embryo. Proc. Soc. Exper. Biol. & Med. 76: 289–292, 1951.

(50) JONXIS, J. H. P., SMITH, P. A., and HUISMAN, T. H. J.: Rickets and amino-aciduria. Lancet 263: 1015–1017, 1952.

Protection of Mice Against X Irradiation by Plasma Proteins[1]

AGNES N. STROUD, AUSTIN M. BRUES, *and* MILDRED M. SUMMERS, *Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.*

In view of the role played by the spleen in hematologic recovery from irradiation injury, as demonstrated by Jacobson and his associates (*1–3*), and the suggestion that the active agent may be of humoral nature, it was thought that prolonged recirculation of blood through an isolated spleen might yield material of therapeutic value. In the course of pursuing this investigation it was found that, while no material in the perfusates of dog spleen was protective to the mouse if injected after irradiation, plasma of the dog and other species, without perfusion, reduced radiation mortality to a considerable extent if given to mice intravenously a few minutes before irradiation (*4–6*). Results of experiments in which plasma or serum were dialyzed or fractionated by ammonium sulfate precipitation indicated that the active material resided in the plasma proteins (*7–11*).

The optimal time of injection of this material is between 3 and 10 minutes before the beginning of irradiation. The effectiveness is reduced if the interval is 15 minutes, and also if the injection is given immediately before the irradiation period, which lasts between 3 and 4 minutes. No effect is measurable if the injection is given one hour or more before irradiation. The preirradiation injections were given slowly by the tail vein, although it was found that the speed of the injection had no influence on the degree of protection; intraperitoneal injections were ineffective.

Materials and Methods

In the routine assay of the materials discussed here, mice were injected intravenously, by tail vein, either 5 minutes before, or within a few minutes after, total-body irradiation. Carworth Farms' CF-1 female mice weighing between 18 and 25 grams, 8 to 11 weeks old, were given a standard X-ray dose of 800 r to the whole body, which approximates the LD_{99} at 17 days, with the median mortality occurring on the 11th day. The conditions of irradiation were: 250 KVP, 15 Ma., using a 0.5 mm. copper plus a 3.0 mm. Bakelite filter, with the HVL equal to 1.8 mm. of copper. The

[1] Received for publication November 8, 1954.

target distance was such as to deliver 220 r per minute; during irradiation, unanesthetized mice were confined, two at a time, in perforated celluloid tubes. In general, several experimental materials were tested, together with X-ray controls, at each time of irradiation. The mice were maintained for the duration of life after treatment.

In order to determine an "apparent dose" for purposes of tabulation, groups of mice were given various smaller amounts of X-ray treatment under the same conditions. Comparison was then made of the survival curves of mice protected against 800 r with those of mice irradiated at lower doses. This comparison was made by inspection of the curves (exemplified in the text-figures) and it can be seen that within the range where most of the values fall, an apparent dose can be ascertained within about 50 r. From this a "dose reduction" can be calculated in percent of the 800 r administered. It is understood that this value for dose reduction applies only to the course of survival within the range of radiation doses administered, and, except where collateral data are presented, does not imply that the course of pathologic events in the treated animal is identical with that in one irradiated by a lower dose without protective treatment.

In the perfusion experiments, dog spleens were perfused for 5 hours at 37° C. under aseptic conditions in a modification of the Carrel-Lindbergh (*12*), Werthessen (*13*), and Hechter (*14*) organ-culture apparatus in which the perfusing media were passed through the organ, usually at a rate of about 50 ml. per minute, under physiological conditions of blood pressure and pulsatile flow (fig. 1). The perfusing fluids were either heparinized blood of the same dog diluted with equal parts of White's nutritive saline medium (*15*) or White's medium alone. The perfusates were centrifuged and the supernatant plasma or medium was then injected into the mice before or after irradiation. In three experiments, the perfused spleen was that of a dog which, one hour previously, had been irradiated (400 r, total body) while the spleen was protected by a lead shield

In preliminary separations, dog plasma was dialyzed against distilled water and then physiological saline through a standard cellophane sac; the precipitates of plasma in half-saturated and saturated ammonium sulfate were similarly dialyzed. Further fractionation of plasma proteins was carried out in this laboratory by Miss Louise Seki, using alcohol precipitation at low temperature. When it was clear that such fractionation afforded promise of concentrating the material, and that human plasma as well as that of many other species was active, purified human plasma protein fractions prepared by Cohn's method (*16*) were obtained from several Public Health and commercial laboratories.[2]

The plasma protein fractions were reconstituted in sterile 0.85 percent NaCl and the *p*H adjusted to 7.4 ± 0.4 for intravenous injection. Mouse serum, obtained from Carworth Farms in lyophilized form, was reconstituted with sterile distilled water.

[2] We are indebted to the Michigan Department of Health, the Massachusetts Department of Health, the American Red Cross, The Laboratory of Physical Chemistry of Harvard University, Difco Laboratories, Detroit, Mich., The Squibb Company, New Brunswick, N. J., Cutter Laboratories, Berkeley, Calif., and Armour Laboratories, Fort Worth, Texas, for generous supplies of the materials tested.

Results

The original perfusion experiments demonstrated that recirculation of autologous blood through the dog spleen (50 to 60 recirculation cycles) yields no protective factor that was not already present in the plasma before perfusion or incubation, and that White's medium perfused through a spleen in the same manner is ineffective. In three separate experiments, in which the spleen had been protected by a lead shield during the irradiation of the donor dog, its perfusion by White's medium resulted in a perfusate which was protective to the same degree as dog plasma. Since this perfusate, like all of the plasmas, sera, and protein fractions tested, failed to influence the course of radiation mortality when administered after irradiation, it was assumed to be unlikely that the specific agent promoting hematopoietic recovery, which is effective after irradiation, was involved. Subsequent experiments have been devoted to an attempt to isolate the active material in plasma or serum. In table 1 it will be seen that pretreatment by the latter perfusate reduces mortality after 800 r to that which ordinarily follows 550 r; dog serum either untreated or perfused through the spleen for 5 hours reduces the apparent dose to 575 r.

Later experiments using homologous and other heterologous plasmas and sera were performed and the results appear in tables 1 and 2, where it is seen that the active factor is also present in rat, mouse, and horse serum. It was found in dog serum as well as in plasma, and in human and rat plasma (not tabulated). Commercially prepared pork and beef plasmas, however, were inert; and the ascitic fluid of mice bearing the Krebs' ascites tumor, although electrophoretically similar to mouse plasma, was inert.

As mentioned above, separated plasma proteins appeared to contain all the activity of plasma and yielded the same "dose reduction" as the original plasma. Fractionation by salting out with ammonium sulfate indicated that the activity was limited to the globulins; this fact was further borne out by the demonstrated ineffectiveness of human- and beef-plasma albumin in amounts equal to those in plasma.

A summary of the effectiveness of each of the major plasma-protein fractions and subfractions tested appears in table 1, which shows the mean survival data and the apparent doses calculated from these. Most of the fractions showed some activity, but the most active ones are seen to be fractions II and III and subfractions III–2 and III–3, in which the apparent dose is reduced to 575 r. In text-figure 1 the survival curves are plotted from the combined data for fractions II and III and compared with the percent survival of mice receiving various doses of radiation. It was noted that different lots of the separated proteins varied in the distribution of the active material through the series of fractions. Table 3 shows the results of separate experiments involving the two main effective fractions, and in table 4 there is a similar treatment of the other fractions and subfractions.

TABLE 1.—*(A) Percent survival and apparent dose resulting from pretreatment of mice given 800 r total-body X rays. (B) Survival after various X-ray doses without pretreatment*

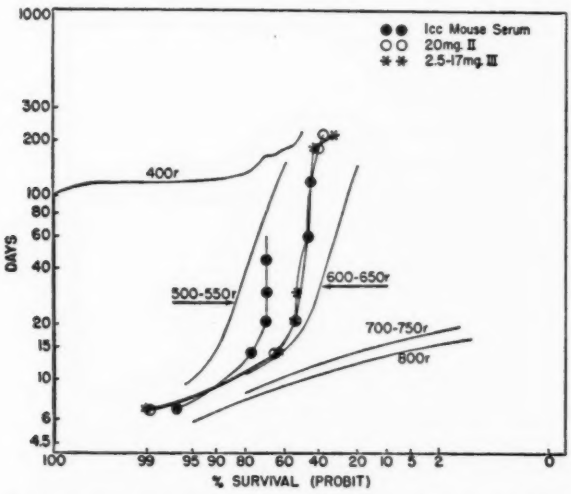
(A) Pretreatment before 800 r	No. mice	Percent survival, weeks								Apparent dose
		1	2	4	8	16	24	32	36	(*r*)
Serum or plasma:										
Mouse; 1 cc	47	98	80	74	74	....	....	....	....	550
Horse; 1 cc	20	95	70	55	50	....	....	....	....	575
Dog; 1 cc	52	79	77	46	46	44	42	25	....	575
Dog (perfused through dog spleen); 1 cc	40	83	83	55	55	55	55	28	....	575
Rat; 0.8 cc	10	100	70	60	50	50	20	10	....	575
Bovine*; 20 mg	10	100	0	....	....	....	....	....	....	800
Porcine*; 20 mg	10	100	0	....	....	....	....	....	....	800
Krebs' ascitic fluid; 1 cc	18	66	0	....	....	....	....	....	....	800
Blood-plasma protein fractions (human):										
I; 8.3–20.0 mg	50	100	28	10	10	4	4	4	....	750
II; 8.3–26.5 mg	66	97	66	53	47	45	41	....	....	575
II–1, 2; 15.0–20.0 mg	18	61	50	50	44	39	39	39	39	600
III; 2.5–17.4 mg	69	99	64	53	51	44	40	34	31	575
III–1; 0.6–4.8 mg	18	66	33	33	33	26	26	21	0	650
III–2; 10.0 mg	10	100	60	50	40	40	40	20	20	575
III–3; 10.0 mg	10	100	60	40	40	40	40	20	10	575
IV–1; 5.0–7.5 mg	19	94	42	36	26	26	26	26	13	650
IV–3, 4; 10.0 mg	10	100	10	0	....	....	....	....	....	800
IV–4; 4.6–39.0 mg	80	90	2	0	....	....	....	....	....	800
Other materials:										
White's medium; 1 cc	20	70	0	....	....	....	....	....	....	800
White's medium (perfused through dog spleen); 1 cc	40	60	8	8	0	....	....	....	....	800
White's medium (perfused through shielded spleen of X-rayed dog); 1 cc	80	89	76	74	59	46	46	46	....	550
Total proteins from perfused plasma; 1 cc	10	80	80	50	50	....	....	10	....	575
Beef-plasma albumin*; 1 cc	10	60	....	....	....	....	....	....	....	800
Bovine *gamma* globulin*; 1 cc	10	90	0	....	....	....	....	....	....	800
Coenzyme A†; 1.7 mg	10	80	50	40	40	40	30	30	....	600
Liver filtrate; 0.5 cc	9	100	22	22	22	22	22	22	11	700
Lysozyme; 16.4 mg	10	80	0	....	....	....	....	....	....	800
Liver-coenzyme conc.; 9.7 mg. (13 Lipmann units/mg.)	10	70	50	0	....	....	....	....	....	800
(B) X ray										
400 r	20	100	100	100	100	100	95	55	....	400
500 r	40	95	93	83	75	75	75	55	....	500
600 r	40	98	60	60	45	43	23	20	....	600
700 r	40	88	5	0	....	....	....	....	....	700
800 r	398	90	4	0	....	....	....	....	....	800

*Armour.
†Pabst.

TABLE 2.—*Percent survival of mice injected intravenously with 1 cc. of homologous or heterologous serum or plasma before total-body exposure to 800 r X rays*

Serum or plasma	No. mice	Percent survival, weeks							Apparent dose (r)
		1	2	3	4	8	24	32	
Mouse*	17	100	90	82	82	82	....	....	550
	9	89	56	56	56	56	....	....	
	20	100	85	75	75	75	60	45	
Mean Percent	.....	98	80	74	74	74	....	....	
Horse†	10	100	70	60	50	40	....	....	575
	10	90	70	60	60	60	....	....	
Mean Percent	.....	95	70	60	55	50	....	....	
Rat‡	10	100	70	60	50	50	20	10	575
Dog: Serum	11	100	90	90	90	90	73	63	575
Plasma	41	73	73	34	34	34	34	15	
Mean Percent	.....	79	77	46	46	46	42	25	

*Lypholized and reconstituted.
†Brought to 60° C., 3 minutes, Difco Laboratory.
‡0.8 cc.



TEXT-FIGURE 1.—Curves showing course of survival in irradiated CF-1 mice (*thin lines*) and in three groups of mice given 800 r after administration of agents as shown. Certain X-ray dosage groups have been combined, since the actual survival curves of the 30 mice employed did not differ significantly between them.

Examination of these tables shows that fractions II and III, as well as the subfractions tested, are generally effective, whereas fraction IV–1 is effective in only two of several separate experiments. Fraction I seems to have slight activity, but only at the higher dosages employed. From table 3, in recapitulating the results with fraction II, we have omitted

TABLE 3.—*Percent survival of mice injected intravenously with various amounts of human-protein fractions II and III (Cohn)* before total-body exposure to 800 r X rays*

Expt. No.	Plasma protein fraction	Mg. injected per mouse	No. mice	Percent survival, weeks								
				1	2	3	4	8	16	24	28	36
1 [4]*	II	26. 5	9	100	56	56	56	22	22	22	22	....
2 [1]	II	20. 0	9	89	56	56	56	45	45	45	45	45
3 [2]	II	20. 0	10	100	70	70	60	60	60	60	50	50
4 [3]	II	20. 0	9	89	33	22	22	22	22	11	11	....
5 [6]	II	20. 0	9	100	89	67	67	67	67	67	67	....
6 [6]	II	20. 0†	10	100	90	70	70	70	60	60	60	....
7 [5]	II	8. 3	10	100	70	40	40	40	40	20	20	0
Mean Percent				97	66	54	53	47	45	41	39	....
8 [6]	III	17. 4	8	100	62	62	62	62	50	50	38	38
9 [4]	III	16. 6	10	100	60	40	40	40	30	30	30	30
10 [6]	III	16. 3	7	100	100	100	100	100	100	86	86	43
11 [6]	III	11. 5	9	100	56	44	44	44	33	33	22	0
12 [6]	III	8. 2	6	100	83	67	67	67	67	50	33	33
13 [3]	III	5. 0	10	100	40	30	30	30	30	30	20	20
14 [4]	III	4. 0	9	89	56	33	33	22	22	22	22	22
15 [4]	III	2. 5	9	100	44	44	44	44	22	22	22	22
Mean Percent				99	64	53	53	51	44	40	34	31

*Human-blood-protein fractions supplied through the courtesy of:
[1] Squibb Co.
[2] Cutter Lab.
[3] Armour Lab.
[4] Harvard Phys. Chem. Lab., Harvard Univ.
[5] Michigan Dept. of Health.
[6] Mass. Dept. of Public Health, Div. of Biological Labs.
†Dialyzed.

one experiment identical with the first one, in which there were no survivors at the end of 2 weeks; and another identical with the fifth one, in which there were no survivors at 3 weeks. It is not known whether these aberrant results can be attributed to statistical fluctuation, biological or chemical variation. A few experiments to determine the loss of effectiveness as the dose is decreased are shown in table 5. This makes it clear that in the case of one preparation the effectiveness diminishes between 5 and 1 mg., whereas a dose of 1 mg. of a different preparation may exert an appreciable effect. The laborious task of working out a dose-dependence curve in further detail has not been attempted.

To investigate inactivation of the factor, fractions II and III, in saline were tested after heating in a boiling-water bath for 10 minutes. It was found that the activity was entirely destroyed. Fraction II was dialyzed against 0.85 percent NaCl for 20 hours in the refrigerator. A flocculent precipitate was discarded and the supernatant was found to be as effective as the starting material (table 3, experiment 6).

Since -SH groups play a part in radiation protection, and coenzyme A has an available -SH group, a crude extraction of coenzymes and other substances from five 10-month-old mouse livers was made by homogenizing at 0° with 1.5 volumes of 0.85 percent NaCl and refrigerating overnight.

TABLE 4.—*Percent survival of mice injected intravenously with various amounts of human-plasma-protein fractions (Cohn)* before total body exposure to 800 r X rays*

Expt. No.	Plasma-protein fraction	Mg. inj. per mouse	No. mice	Percent survival, weeks								
				1	2	3	4	8	16	28	36	48
1 [5]*	I	20. 0	10	100	40	20	10	10	10	10	10	10
2 [2]	I	20. 0	10	100	60	20	20	10	10	10	10	0
3 [1]	I	15. 6	10	100	38	11	11	0	....	....	....	....
4 [3]	I	10. 0	10	100	0	....	....	....	....	....	....	....
5 [5]	I	8. 3	10	100	0	....	....	....	....	....	....	....
6 [5]	II–1, 2	20. 0	10	100	60	50	50	50	40	40	40	20
7 [5]	II–1, 2	15. 0	8	100	63	50	50	38	38	38	38	12
8 [2]	III–1	4. 8	10	100	70	40	40	40	40	30	30	0
9 [2]	III–1	0. 6	8	100	63	25	25	25	12	12	12	0
10 [4]	III–2	10. 0	10	100	60	50	40	40	40	20	20	0
11 [4]	III–3	10. 0	10	100	60	40	40	40	40	20	10	0
12 [1]	IV–1	20. 0	10	100	0	....	....	....	....	....	....	....
13 [5]	IV–1	15. 0	10	100	0	....	....	....	....	....	....	....
14 [6]	IV–1	11. 7	10	100	0	....	....	....	....	....	....	....
15 [5]	IV–1	7. 5	9	89	56	34	23	23	23	23	11	0
16 [2]	IV–1	5. 0	7	100	29	29	29	29	29	29	15	15
17 [1]	IV–1	4. 0	10	100	0	....	....	....	....	....	....	....
18 [1]	IV–1	2. 5	10	80	0	....	....	....	....	....	....	....
19 [1]	IV–3.4	10. 0	10	100	10	0	....	....	....	....	....	....
20 [6]	IV–4	39. 0	10	100	0	....	....	....	....	....	....	....
21 [4]	IV–4	26. 5	10	90	0	....	....	....	....	....	....	....
22 [1]	IV–4	20. 0	10	100	10	....	....	....	....	....	....	....
23 [6]	IV–4	20. 0	10	100	0	....	....	....	....	....	....	....
24 [4]	IV–4	20. 0	10	80	0	....	....	....	....	....	....	....
25 [3]	IV–4	15. 3	10	100	10	0	....	....	....	....	....	....
26 [5]	IV–4	15. 0	10	70	0	....	....	....	....	....	....	....
27 [1]	IV–4	4. 6	10	80	0	....	....	....	....	....	....	....

*Human blood protein fractions supplied through the courtesy of:
[1] Squibb Co.
[2] Cutter Lab.
[3] Armour Lab.
[4] Harvard Phys. Chem. Lab., Harvard Univ.
[5] Michigan Dept. of Health.
[6] Mass. Dept. of Public Health, Div. of Biological Labs.

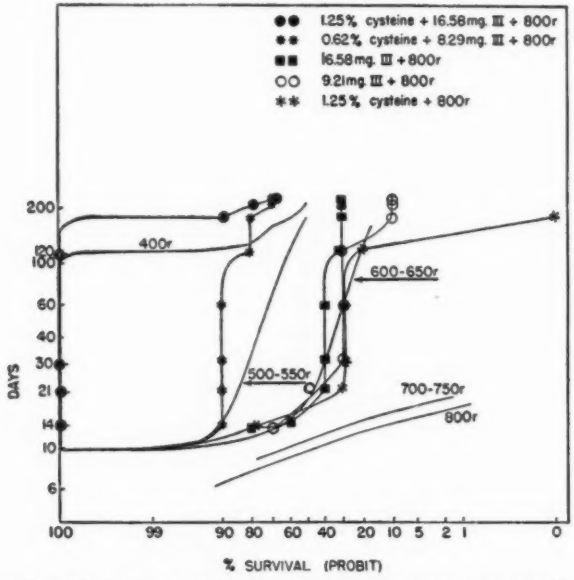
TABLE 5.—*Effectiveness and dose-dependence of plasma fraction III* in minimal doses: Survival of mice treated prior to 800 r X rays*

Expt. No.	Plasma-protein fraction	Mg. inj. per mouse	No. mice	Percent survival, weeks					
				1	2	3	4	6	7
1 [1]*	III	1. 0	10	100	40	10	10	10	0
2 [2]	III	1. 0	20	95	5	0	.....	.....	.....
3 [2]	III	3. 0	20	80	5	5	.....	.....	.....
4 [2]	III	5. 0	20	95	25	15	15	10	0

*Human-blood-protein fractions supplied through the courtesy of:
[1] Squibb Co.
[2] Mass. Dept. of Public Health, Div. of Biological Labs.

The homogenate was centrifuged and 0.5 ml. of the filtrate was tested for activity. A liver coenzyme concentrate containing 13 Lipmann units of coenzyme A per mg., and a purified coenzyme A (Pabst), probably not more than 75 percent pure, were also tested. The results are shown in table 1. The crude-liver filtrate showed a slight degree of activity, whereas coenzyme A injected in an amount of 1.7 mg. reduced the apparent dose to 600 r, and the liver concentrate was found to be ineffective in the dose used. The activity of coenzyme A in the liver concentrate injected was estimated to be about one-tenth that of the 1.7 mg. of the purified coenzyme. Bacq (*17*) found that 4 mg. of coenzyme A is toxic to C57 mice, but after this dose 3 of 8 mice survived a dose of 700 r X radiation; survival after treatment with 3 mg. before irradiation was similar, while only one of 10 X-irradiated controls survived. Since lysozyme is rich in disulfide crosslinks and other components of possible importance, it was similarly tested (table 1) but found ineffective.

In relation to the question of sulfhydryl protection (*18*), the sulfhydryl content of active samples of dog plasma was measured by Dr. H. M. Patt and found to be far below that required for a significant protection. Experiments were done to test additivity of cysteine and fraction III; results are shown in table 6 and text-figure 2. It is seen that fraction III markedly reinforces the action of a near-maximal dose of cysteine, and that additivity exists under all of the conditions employed. No evidence of toxicity was seen during or after injections of the two substances together.



TEXT-FIGURE 2.—Actual survival curves of mice protected against 800 r X rays by combinations of cysteine and human-plasma-protein fraction III, compared with survival after X irradiation alone (see table 6).

TABLE 6.—*Percent survival of mice injected intravenously with blood-protein-fraction III (Cohn) and cysteine before total-body exposure to 800 r X rays (1 ml. injected)*

Nature of experiment	No. mice	Percent survival, weeks									Apparent dose (r)
		1	2	3	4	8	16	24	28	32	
9.21 mg. III+800 r	10	100	50	30	30	30	30	10	10	10	625
16.58 mg. III+800 r	10	100	60	40	40	40	30	30	30	30	575
0.62% cysteine+8.29 mg. III+800 r	10	100	90	90	90	90	80	80	70	70	475
1.25% cysteine+16.58 mg. III+800 r	9	100	100	100	100	100	100	89	78	67	<400
1.25% cysteine+800 r	10	100	80	30	30	30	30	20	0	0	650
800 r	10	100	0	....	....	....	....	....	....	....	800

To explore the question of whether injections of the factor might, through some physiological mechanism, induce anoxia, 10 mice injected with fraction III and 10 control mice were placed in a desiccator which was ventilated with nitrogen. All of the mice lost consciousness during the same period of time, and all subsequently recovered together when removed from the desiccator.

All animals to which protective substances were given were kept under observation until death and the partially protected animals were compared with unprotected mice that had received smaller X-ray dosages. Ocular opacities were first seen by visual observation as early as 4 months after irradiation in mice pretreated by these materials and given 800 r. One or both eyes are completely opaque by the seventh month in these animals. On the other hand, mice given the "apparent dose" alone (500–600 r) showed macroscopically no visible opacities at the end of 7 months. In the case of the experiments shown in table 6, all of the 7-month survivors of fraction III plus 800 r show complete opacities, whereas those receiving pretreatment with cysteine in addition are not visibly affected. Thus, we have no evidence that the lens shares in the protective action of the plasma protein, although the additional protection afforded by cysteine does seem to include the lens.

Partial loss of hair is apparent after several months in the protected mice, and their appearance is similar to that of mice receiving lower dosages of total-body X radiation or in senile untreated mice. Neoplasms have not been tabulated, but they do not appear to be more frequent in protected mice than in those given sublethal dosages of X radiation. Weight changes after irradiation in mice receiving 1 ml. of mouse serum plus 800 r are similar to those in mice receiving 550 r without the factor in addition. Control mice receiving 600 r lost weight more rapidly than the pretreated 800 r mice.

Mice protected by serum show a decline in total white blood cells for the first 7 to 9 days similar to the decline seen in unprotected mice. This decline was followed by recovery in about 3 weeks. Direct comparison with mice receiving 550–600 r has not been made.

The spleen weight response has been followed in a series of mice pretreated with mouse serum and then irradiated with 800 r. The weight decreases by 70 to 80 percent in 4 days, as in the 800 r control mice. In the pretreated mice, weight gain begins at 7 days and by the 17th day is greater than that of the spleen before treatment by a factor of two, as is the case with mice treated with 550 r alone. Gross and histologic examination of the recovering spleens after 12 to 15 days shows a corrugated appearance due to active foci of regeneration with a high degree of mitotic activity.

Those mice that succumb after partial protection have shown gross changes characteristic of acute radiation disease: edema of the soft tissues of the head and neck, petechiae in the lungs and intestinal tract, and weight loss up to 40 percent of the body weight.

In almost all of the experiments, including representative experiments in which all the substances described here have been tested, an equal number of mice have been injected intravenously shortly after irradiation. In the case of dog plasma an additional injection has been given 24 hours after irradiation. In no case has there been any evidence of postirradiation therapeutic effect: all mice so treated died within 18 days.

To examine the possibility that the response to the material given before irradiation might be peculiar to the mouse, 8 rats weighing 120 to 130 grams have been given intravenous injections of 5 ml. of mouse serum before irradiation (800 r). Twenty-day survival was 62 percent, whereas mortality was 100 percent in 10 days in rats irradiated without pretreatment.

Dog plasma and human-fraction II were administered prior to the irradiation of mice with 1,000 r. The time-survival curves were compared with those of mice given lower doses of X radiation, and showed "apparent doses" of 650 and 800 r, respectively. From this a "dose reduction" from 1,000 r of 35 percent and 20 percent is seen. Dose reductions from 800 r by the same two agents, calculated from table 1, are 31 percent and 28 percent. The protective effect has not yet been examined with respect to other X-ray doses.

Discussion

The data presented here show that CF-1 female mice treated just prior to irradiation with homologous and heterologous plasma and serum, spleen-perfused sera, and specific protein fractions from human serum, are partially protected against a whole-body radiation dose of 800 r, responding with a survival pattern like that of animals given about 550 r. The same materials given just after irradiation have no effect.

Separation of the plasma proteins shows that the active factor is confined to the globulins. The factor is not cleanly separated by alcohol precipitation methods, although it appears most abundantly in fractions II and III and their subfractions: fraction I shows less activity and some preparations of fraction IV–1 have shown activity. From this it does

not appear that the material corresponds to one of the major electrophoretic components of plasma, since none of them seem to be distributed correspondingly through these fractions. The equivalence of fractions II and III, as shown in table 3, seems to indicate that it is not the same as immune *gamma* globulin, and in fact one pair of preparations included a fraction II which is prepared by the Massachusetts Department of Public Health for use in protection against poliomyelitis, and a fraction III which is discarded in its preparation. The smallest amount of any material that has shown a significant degree of protective activity is 0.6 mg. of fraction III–1. Exhaustive examination of dosage-response relations in the various fractions, however, has not been possible because of limitations on the amounts of material available.

Not much can be said about the physiological mode of action of the material. Although large amounts of plasma have been used for injection (in some cases approaching the blood volume of the mouse), it is clear from the fact that albumin in equivalent amounts is totally ineffective and that some globulin fractions are effective in very small amounts, that the change in blood volume following injection cannot be considered responsible for the decreased response of the mouse to irradiation.

It has not been possible to correlate other physiological changes in the injected animals with the effectiveness of the treatment. It has been observed on several occasions that the rapidity of the injection bears no relation to subsequent survival after irradiation. Injection of some substances has given rise to collapse of brief duration, but this has been true of both active and inactive preparations, and it has been noted that those animals that respond in this way (whether due to speed of injection or individual susceptibility) are no more likely to survive the irradiation than those that do not. That a general anoxic state induced by the injection is responsible for protection seems unlikely, also, because injections of fraction III have not potentiated the toxic effects of anoxia induced by lowering the O_2 tension of ambient air. It is hardly necessary to mention that concentration of the reducing substances in plasma protein is far below that which has any effect on subsequent irradiation; in addition, the albumin fraction contains the major proportion of the sulfhydryl groups and is ineffective.

While the term "dose reduction" has been used here, it is not intended to imply that all of the mechanisms of radiation injury are affected to an equivalent degree. Such an implication is certainly not true in the case of cataracts, where no dose reduction is apparent. It may be said that a group of animals protected by 1 ml. of serum or plasma is quite comparable with mice given 550–600 r and otherwise untreated, with respect to weight, hematologic effects, gross early and delayed pathologic responses, and the survival curve. There is some evidence that the relative degree of protection is the same where the mice receive 1,000 r.

Concerning the body-weight response, it appears that the situation is different where factors promoting hematologic recovery are employed. Mice protected against 800 r by spleen homogenate have been reported

(*19*) to lose weight at the same rate as untreated mice given 800 r for the first nine days, after which recovery occurs rapidly. Mice protected by plasma protein, on the other hand, fail at any time to show the steep loss of weight seen in those not so protected (*10*). It can be said that our data are insufficient, as are published data which could be used for comparative purposes, to establish whether or not we are dealing with a protection of specific physiological mechanisms.

The fact that none of the preparations, in the amounts used, reduced the "apparent dose" below 500 r may be of significance. It may be taken to suggest either that they act upon a single component of radiation mortality, or that they exert a physiological effect which is fully in operation where mortality is only partially relieved. The additivity between the effect of these materials and the reaction to cysteine seems to indicate that the two operate through different pathways.

Summary

Intravenous administration of serum or plasma, homologous or heterologous, or of plasma proteins to mice a few minutes before irradiation afford partial protection against death and other radiation effects. With the exception that no protection against cataracts is apparent, the survival pattern and pathologic changes of protected animals correspond to those of animals given lower radiation dosage. It has not been possible to reduce the mortality after 800 r below that of unprotected mice after 550 r.

Using fractionated-plasma proteins it is found that albumin is entirely inactive; the greater part of the activity is found in fractions II and III of Cohn. It appears not to be identical with immune *gamma* globulin and has not been identified with any major electrophoretic component.

The effect cannot be explained on the basis of the sulfhydryl content of plasma proteins, nor by blood volume changes resulting from injection. Evidence has been presented which makes it appear doubtful that shock or an anoxic state induced by injection is primarily responsible for the protective action. Additivity of an apparently saturated response with that of cysteine suggests that the action takes place through a different pathway from cysteine action.

None of the materials examined has any effect on radiation mortality if administered immediately after irradiation.

Perfusion of White's medium through a dog spleen which has been shielded while the dog was given a lethal dose of total-body X radiation yields a material in the perfusate with similar action; perfusion of a normal dog spleen with the same medium fails to do so.

Bibliography

(*1*) Jacobson, L. O., Simmons, E. L., Marks, E. K., Robson, M. J., Bethard, W. F., and Gorton, E. O.: The role of the spleen in radiation injury and recovery. J. Lab. & Clin. Med. 35: 746–779, 1950.

(*2*) Jacobson, L. O., Simmons, E. L., Marks, E. K., and Eldredge, J. H.: Recovery from radiation injury. Science 113: 510–511, 1951.

(3) JACOBSON, L. O., SIMMONS, E. L., MARKS, E. K., GASTON, E. O., ROBSON, M. J., and ELDREDGE, J. H.: Further studies on recovery from radiation injury. J. Lab. & Clin. Med. 37: 683–697, 1951.

(4) JACOBSON, L. O.: Evidence for a humoral factor (or factors) concerned in recovery from radiation injury: *A review.* Cancer Res. 12: 315–325, 1952.

(5) STROUD, A. N., and HASTERLIK, R. J.: Progress report: Organ Culture. Argonne National Laboratory ANL–4571, p. 63, 1951.

(6) STROUD, A. N., HASTERLIK, R. J., and BRUES, A. M.: Progress report: Organ Culture. Argonne National Laboratory, ANL–4676, pp. 60–62, 1951.

(7) STROUD, A. N., and BRUES, A. M.: Protection of mice against irradiation by dog plasma and spleen perfusates. (Abstract.) Fed. Proc. 11: 155, 1952.

(8) ———: Plasma protein fractions protecting mice against irradiation. Fed. Proc. 13: 546, 1954.

(9) ———: Monthly Progress Report: Argonne National Laboratory, ANL–5200, p. 17, 1954.

(10) ———: Effect of various protective substances on survival and weight loss in irradiated mice. Argonne National Laboratory, ANL–5288, p. 85, 1954.

(11) ———: Protection of mice against irradiation by dog plasma and spleen perfusate. Argonne National Laboratory, ANL–4794, p. 66, 1952.

(12) CARREL, A., and LINDBERGH, C. A.: The culture of organs. New York, Paul B. Hoeber, Inc., pp. 1–221, 1938.

(13) WERTHESSEN, N. T.: A technique of organ culture for protracted metabolism studies. Endocrinology 44: 109–126, 1949.

(14) HECHTER, O.: Lymphocyte discharge from the isolated rabbit spleen by adrenal cortical extraction. Endocrinology 42: 285–306, 1948.

(15) WHITE, P. R.: Cultivation of animal tissues in vitro in nutrients of precisely known constitution. Growth 10: 231–289, 1946.

(16) COHN, E. J., *et al:* A system for the separation of the components of human plasma. J. Am. Chem. Soc. 72: 465–474, 1950.

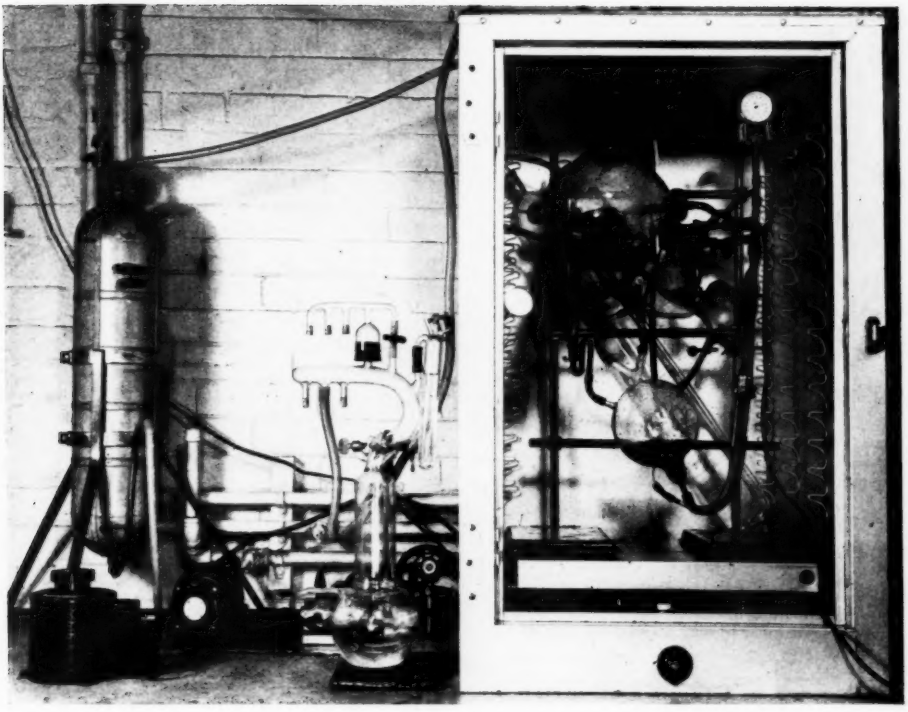
(17) BACQ, Z. M., and HERVE, A.: Coenzyme A et radiations ionisantes. Arch. intern. physiol. 61: 434–436, 1953.

(18) PATT, H. M., MAYER, S. H., STRAUBE, R. L., and JACKSON, E. M.: Radiation dose reduction by cysteine. J. Cell. & Comp. Physiol. 42: 327–341, 1953.

(19) COLE, L. J., FISHLER, M. C., ELLIS, M. E., and BOND, V. P.: U. S. Naval Radiological Defense Laboratory, USNRDL–339, 1952.

PLATE 66

FIGURE 1.—Photograph of organ-culture apparatus used for perfusions. The section for maintaining pulsatile pressure is on the left, outside the incubator. The organ is cannulated and placed in the upper vessel in the incubator; the lower vessel is the reservoir for the perfusing medium.



Stroud *et al.*

320174—55— 27

A Comparative Analysis of Radiation Lethality in Mammals Exposed at Constant Average Intensity for the Duration of Life[1]

GEORGE A. SACHER, *Division of Biological and Medical Research, Argonne National Laboratory, Lemont, Ill.*

1. Introduction

The objective of this paper is to systematize our empirical knowledge about the relationship between dosage and survival time for animal populations exposed to a constant average intensity of ionizing radiations for the duration of life. At present there is substantial information about 7 mammalian species, and for some of these species the intensities investigated range from 0.1 r per day up to 10^7 r per day. The very considerable amount of information available is, however, in fragmentary form because many of the investigations were limited in scope and size. Not until the various sets of data have been reduced to uniform units and expressed in dimensionally appropriate terms can they be adequately compared and analyzed. This review undertakes to show that the existing data on radiation lethality fall into a pattern of impressive simplicity and coherence, and reveal interesting implications for comparative physiology and for the physiology of radiation injury and recovery.

Although the primary objective is to examine the empirical evidence, the examination is conducted with reference to the theoretical as well as to the applied aspects of the subject. One theoretical aspect is the conceptual framework in terms of which the present data acquire significance. A second is the mathematical analysis needed for this essentially quantitative subject. These are developed briefly in the succeeding paragraphs. It will be shown that the most important applications require a well developed theoretical and biometrical apparatus.

With regard to the role that the analysis of lethality data can play in radiobiology and radiation medicine, it may be asserted that lethality data *in themselves* are of limited value. Thus, while it is possible to state mathematical relations between radiation dose and survival time, these "laws" are usually inaccurate even for the data on which they are based, and would in some cases be downright dangerous if extrapolation from them were used in medical practice. More serious is the fact that the parameters of such curve-fitting equations cannot be given an operational

[1] Received for publication November 8, 1954.

Journal of the National Cancer Institute, Vol. 15, No. 4, February 1955
320174—55—28

interpretation, and hence the equations can have little fruitful consequence for physiologic research.

The problem of accounting for dosage-lethality relationships must therefore be approached by first accounting for lethality in terms of the measurable behavior of the living organism. One is then faced with two independent problems, that may be formulated as follows: first, to investigate the statistical relation between physiologic injury and rate of mortality; and second, to give an adequate description of the kinematics of radiation injury to the elementary physiologic units, especially the cellular units. This reduces the general problem to terms that are intelligible and that make possible an operational attack. However, when the problem is thus reduced to basic terms its difficulty becomes evident. The relation of lethality (either as a probability or as a survival time) to dose cannot be less complex than the physiologic responses of the irradiated animal.

In what follows, the available survival data are examined in terms of the hypothesis that the mortality in an interval is a function of a weighted sum of the intensities of physiologic injury in the same interval. With this hypothesis and some unavoidable assumptions we construct a linear model of the radiation injury process, and thence deduce a simple mathematical expression that gives an approximate measure of the postulated injury function.

The operation that is carried out is a transformation in the mathematical sense; the survival data are carried into a form in which they should correspond to directly measured curves of physiologic injury. No attempt is made herein to fit mathematical equations to these curves. The emphasis is rather on comparisons between species and between radiation qualities, and on comparisons of the deduced curves with some known physiologic responses of the various species. It is in this spirit that the task set in the opening sentence is carried out.

2. Derivation of the Relation Between Survival Time and the Underlying Physiologic Injury Process

The fundamental hypothesis (*1*) states that exposure to a dose of ionizing radiations initiates in organisms a *lethal injury process* that is a weighted sum of the several kinds of physiologic injury produced. The hypothesis is developed under the simplifying assumption that the organism responds to radiations in linear fashion (*1*). This implies that: 1) after a brief (impulse) exposure to radiation the lethal process follows a characteristic time course; 2) if only the dose is altered, the lethal process is altered in amplitude but not in form; and, 3) the response to a temporal sequence of like impulses is equal to the sum of the responses to the impulses delivered separately.

The chances of survival of an organism depend on the value of a quantity called the Lethal Injury Function, X, and defined as the sum of the values

of the physiologic variables, x_i, with each given a weight, w_i, according to its importance for survival:

$$X = \sum_{i=1}^{n} w_i x_i \qquad [1].$$

Following a brief exposure to ionizing radiations delivered at time $\tau = 0$, each physiologic variable undergoes a characteristic injury process that at time t has the value

$$x_i(t) = k_i \phi_i(t)\, I\, d\tau \qquad [2]$$

where $\phi_i(t)$ is the time-response to unit dose for the i^{th} variable,
$I\, d\tau$ is the quantity of irradiation delivered at intensity I in time interval $d\tau$, and
k_i is the dose-sensitivity constant for the i^{th} variable.[2]

The injury to the i^{th} variable at time t after a brief exposure at time τ $(t > \tau > 0)$ is

$$x_i(t,\tau) = k_i \phi_i(t - \tau) I\, d\tau \qquad [3].$$

The value of the lethal process following an impulse dose at time $\tau = 0$ is therefore

$$X(t,\tau) = -\, I\, d\tau \sum_{i=1}^{n} w_i k_i \phi_i(t - \tau) \qquad [4].$$

For a series of impulses delivered with a constant interval, h, such that $\tau_j = jh$

$$X(t) = \sum_{i=1}^{n} \sum_{j=0}^{m} w_i k_i \phi_i(t - \tau_j) I d\tau \qquad [5].$$

In the limiting case of continuous exposure this sum becomes an integral

$$X(t) = -I \int_0^t \sum_{i=1}^{n} w_i k_i \phi_i(t - \tau) d\tau \qquad [6].$$

The intensity variable has been brought outside the integral because we are herein concerned only with the case $I =$ constant for $\tau \geq 0$.[3]

The system-specific weighting coefficients w_i and k_i cannot be evaluated in application to the whole-body external irradiation problem, so we have no alternative but to suppress the subscripting and discuss only the summed response. Equation [6] may then be rewritten

$$X(t) = -\, I \int_0^t \phi(t - \tau) d\tau = I \int_0^t \phi(\tau) d\tau \qquad [7].$$

In equation [7] the summed injury response is written as a deviation from a baseline of zero, which is equivalent to taking as origin the normal

[2] If it should become necessary to compare different radiation qualities or other noxae that have quantitatively different actions, the k's would require a second subscript for the specific agent denoted.

[3] The case of exponentially decreasing dose rate, $I = I_0 e^{-\alpha t}$, has also been solved, and applied to the lethality of injected tritium (*8*).

physiologic state at time zero. However, this introduces the incorrect implication that the normal state is independent of time (age) thereafter. The progressive change in physiologic state that accompanies, and in a sense is, the aging process is taken into account in the following fashion (*1*). Consider that the physiologic changes due to age and to irradiation accumulate independently and are additive (*3*), so that the aging process is represented by an additive term:

$$X(t) = A\,(t) + I\int_0^t \phi(\tau)d\tau \qquad [8].$$

The trend of the aging process for many physiologic systems is a linear function of age to a first approximation (*4*), so we may write for the aging function from an origin at the onset of exposure

$$A\,(t) = \beta t \qquad (\beta = \text{constant}) \qquad [9].$$

For a given I in equation [8] the function $X\,(t)$ becomes equal at time t^* to a value M, called the lethal bound of injury (*1*). We define M as the value attained by the Lethal Injury Function, X, at the time of death, t^*, of a representative member of the treated population,[4] usually chosen to be the mean or median animal. It must be assumed in the absence of further information that the value of M is independent of age, species, toxic agent employed, etc. (see Sec. 4.4). We now define the Cumulant Lethality Function, C_L, as

$$C_L(t^*) = \frac{1}{M}\int_0^{t^*} \phi(\tau)d\tau \qquad [10].$$

This is seen to be the integral of the Lethal Injury Function for unit dose rate with the Lethal Bound as the unit of scale. Now let t_0 be the survival time of nonirradiated animals under the same condition. We have from equation [9] and the definition of M

$$\beta t_0 = M \qquad [11].$$

Now in equation [8] let $X\,(t)$ reach the lethal bound M

$$M = I\int_0^{t^*} \phi(\tau)\,d\tau + A(t^*) \qquad [12],$$

so that

$$\frac{1}{I}\left[1 - \frac{A(t^*)}{M}\right] = C_L \qquad [13].$$

However, by using equations [9] and [11],

$$\frac{A(t^*)}{M} = \frac{\beta t^*}{\beta t_0} = \frac{t^*}{t_0} \qquad [14]$$

[4] The foregoing definition of the lethal bound is an oversimplified representation of the death process, for it neglects the stochastic nature of the process. This definition is, however, better suited to the discussion of heterogeneous small sample data such as we are here concerned with than is a more ponderous exact treatment. The stochastic aspects of the death process can best be deduced and exhibited in application to data from large population, for which the actuarial functions can be calculated. Such development and application are reserved for a following communication.

where t_0 is the length of life of the representative member of the non-irradiated population. We obtain finally the solved form of the Cumulant Lethality Function

$$C_L(t^*) = \frac{1}{I}\left(1 - \frac{t^*}{t_0}\right) \qquad [15].$$

Thus it is found that, with the assumptions employed, the physiologic injury function that underlies lethality has a value at time t^* given by the reciprocal of the dose rate required to cause the death of the representative member of a population at time t^*, modified by an aging term that is a linear function of t^*. Where $t^* \ll t_0$, C_L reduces to the reciprocal of the dose rate. If I is a function of time the method of solving for C_L is different (*2*).

3. Data and Computations

Relevant information about species, strain, sex, radiation quality and protraction, daily dosages and treatment pattern are tabulated for each investigation in table 1.

All survival data at 0.1 r per day and some data at higher daily dosages (see table 1) are excluded because they do not differ significantly from control survivals. The after-survival of controls, t_0, presented difficulty in some cases. Satisfactory data are not available for dog, monkey, or rabbit, in particular. One of four procedures was used to estimate control survival, as follows:

1) Use control data as reported;
2) Combine control survival and survival at nonsignificant treatment level to estimate a pooled control;
3) Use information from other sources to estimate control survival (used only where t^* is less than half the estimated t_0);
4) Use a modified formula that permits substitution of survival of treated group for control survival; for monkey (Sec. 4.10) and rabbit (table 1).

The procedure is indicated in table 1 for each experiment, and the adopted numerical values are tabulated. In the great majority of cases, either the experiment included an adequate control group or the experimental survival times were so short that the term t^*/t_0 could be neglected.

There are other sources of uncertainty with magnitudes that can only be guessed at. Variability in calibration of X-ray dosimeters may have a range in excess of 10 percent. *Gamma*-ray dosages probably vary still more widely, because of divergent practices with respect to ensuring equilibrium. To these must be added discrepancies due to divergent calibration geometries.

4. Application of the Cumulant Lethality Transformation to Existing Survival Data

4.1 *Introduction:* In this section we examine the data catalogued in Section 3, as transformed by equation [15] of Section 2. All known data

TABLE 1.—*Catalogue of investigations of survival of laboratory mammals exposed to X rays, gamma rays or neutrons for the duration of life*

Reference	Strains	Quality	Protraction	No. exposures per week	Daily dose range	No. expt. points	Control survival
			MOUSE				
Evans (*5*)	Swiss	n*	B†	5	0.01–7	5	475 C‡
" (*5*)	CF#1	n	B	5	0.01–1.0	4	420 C
" (*5*)	Swiss	X§	B	5	57	1	475 C
Henshaw (*6*)	C3H	X	B	5	5–80	8	322 C
" (*7, 8*)	CF#1	n	B	6	0.12–13	4	434 C
" (*7, 8*)	CF#1	γ‖	B	6	1–8.6	2	420 C
" (*7, 8*)	ABC hybrid	n	B	6	1.2	1	385 C
" (*7, 8*)	ABC hybrid	γ	B	6	8.6	1	385 C
" (*7, 8*)	C58	n	B	6	1.2	1	350 C
" (*7, 8*)	C58	γ	B	6	8.6–17	3	350 C
" (*9*)	CF#1	γ	contin.	.....	5×10^6	1	— — —
Lorenz (*10*)	LAF_1 hybrid	γ	8 hr.	7	0.11–8.8	5	725 C + 0.11
" (*11*)	$C3H_f$	γ	8 hr.	7	4.4–8.8	2	716 C
Quastler (*12*)	C57BL	X	B	7	50–460	9	600 E¶
" (*12*)	C57BL	20 Mv	B	7	100–460	2	600 E
Rugh (*13*)	CF#1	X	contin.	.....	2–5 ($\times 10^6$)	2	— — —
Sacher (*1*)	ABC hybrid	X	B	6	20–1,000	12	538 C
" (*1*)	CF#1	X	B	6	40–200	5	425 C
Smith (*14*)	CF#1	X	contin.	.....	6.5×10^5	1	— — —
Grahn (*15*)	LAF_1 hybrid	γ	16 hr.	7	140–300	4	700 E
Thomson (*16*)	CF#1	γ	24 hr.	7	90–140	3	425 E
			RAT				
Boche (*17*)	Wistar	X	B	6	0.1–10	4	600 C
Hagen (*18*)	Sprague-Dawley	X	B	6	12.5–500	7	500 E
Thomson (*16*)	Sprague-Dawley	γ	24 hr.	7	20–120	6	485 C
Smith (*14*)	Sprague-Dawley	X	contin.	.....	6.5×10^5	1	— — —

			RABBIT				
Boche (*17*)	Wistar	X	B	6	0.1–10	4	415 C + 0.1 + 1.0
Lorenz (*10*)		γ	8 hr.	7	0.11–8.8	5	1,450 1.1 + 2.2
Sacher (*19*)	Swift	X	B	6	40–325	8	1,000 E
T'ang (*20*)		X	B	7	10–50	4	1,000 E
			DOG				
Boche (*17*)	Mongrel	X	B	6	0.1–10	6	3,600 E
Ducuing (*21, 22*)	Mongrel	X	B	7	25–100	2	— — —
Prosser (*23*)	Mongrel	X	B	7	12.5–50	4	3,600 E
			GUINEA PIG				
Lorenz (*10*)	Hybrid	γ	8 hr.	7	0.11–8.8	5	1,280 C
Sacher (*24*)		X	B	3	26	1	— — —
Thomson (*16*)		γ	24 hr.	7	15–120	5	1,000 E
			MONKEY				
Boche (*17*)	Rhesus	X	B	6	0.1–10	4	350 0.1
			BURRO				
Rust (*25–27*)		γ	1/2–20	7	25–1,000	6	— — —

*n = Neutron daily dosages in "n" units.
†B = Brief.
‡C = Specific control data.
§X = Daily doses in roentgens.
‖γ = Daily doses in roentgens.
¶E = Survival estimated from other sources.

are plotted in text-figure 1, in which the value of the lethality cumulant, C_L, for a particular treatment group is plotted against its mean survival time, t^*. Because both variables range over many orders of magnitude, they are plotted on log-log coordinates.

Each species has a distinguishing symbol, as indicated by the key in the figure. Data from X ray, *gamma* ray and neutron exposure are included in the figure, and are given distinguishing symbols, also indicated in the key. The C_L values for neutron exposures are given in rem[5] units, obtained by multiplying each daily neutron dose by the single-dose *RBE* reported by that investigator. The single-dose *RBE* for neutrons with respect to X rays is defined as

$$RBE = \frac{X\text{-ray } LD_{50}}{\text{neutron } LD_{50}}.$$

The *X*-ray LD_{50} is measured in roentgens. The neutron dosages were measured in arbitrary "n" units, but when multiplied by the *RBE* this arbitrary factor is canceled out. This topic is discussed further below (Sec. 4.9).

The *gamma*-ray dosages were all treated as if there were a constant *gamma*-ray *RBE* of 0.5 (see Sec. 4.8), so that the C_L in terms of *gamma*-ray roentgens can be obtained from the values in text-figure 1 by dividing these values by 2.

Before going on to the main tasks of physiologic correlation and species comparison we might consider whether the C_L *versus* t^* plot provides a good basis for these tasks. There are four points that would recommend this representation, quite aside from its theoretical justification. They are:

1) The abscissa is a physical variable, time from the beginning of treatment, that has the same significance for all species.

2) The ordinate is also a physical variable with dimensions $(\text{r/day})^{-1}$ (so that C_L is the injury per unit dose rate).

3) Direct comparison of radiation specific effects is made possible by the correction for natural aging in the various species. The order observed in the forms of the cumulant radiation lethality functions of different species would not be apparent if raw survival data were used.

4) The cumulant function is an empirical function: no arbitrary constants enter into its estimation.

It must be remembered, however, that approximations have been made in the theoretical development, and that insufficient control data and certain experimental difficulties introduce errors that become more serious as t^* approaches t_0.

4.2 *General characteristics of the cumulant functions of the seven species*:— The first point to note is that each species manifests a remarkably tight pattern. Nowhere do the combined X- and *gamma*-ray C_L values for a species at a given survival time have more than a twofold range of variation. This is evidence of a high order of consistency in the results when

[5] Roentgen equivalent mammal.

one considers that, in our laboratory, mouse strains have been found to vary by a factor of 1.3 in single dose LD_{50} and by a factor in excess of 1.7 for C_L in the 50- to 90-day region (*15*). In view of the latter finding it is likely that the greater part of the intraspecies variation seen here will be found to arise from strain differences.

The intraspecies variation is small in comparison with the interspecies differences. In the most extreme case, guinea pig is tenfold more sensitive than rat or rabbit in the 200-day region. No two species present cumulant lethality functions identical in every respect. The two most closely related species taxonomically, mouse and rat, are most nearly alike in the characteristics of their cumulant functions.

These observations justify the construction of cumulant curves for each species by passing curves through the clusters of points, even where the points in different parts of the curves come from different investigators and strains, and inhomogeneity between the X-ray and adjusted *gamma*-ray points, arising from inaccuracy in the provisional estimate of the *gamma* ray *RBE*, must be ignored.

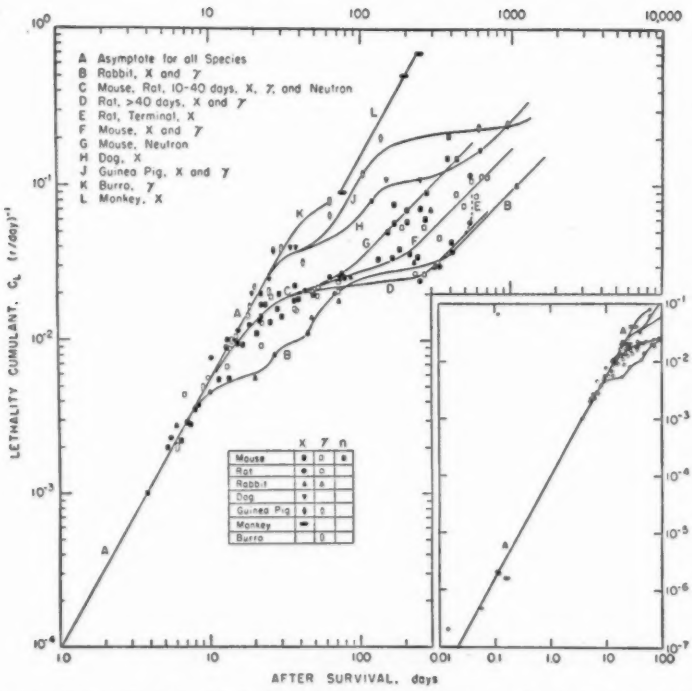
4.3 *Generic features of the cumulant curves:*—Those species for which the cumulant is adequately mapped show in the plot of log C_L *versus* log t^* a steep linear rise at the shortest survival times followed by a plateau period in which the value of C_L is almost independent of time. This horizontal branch is succeeded at later times by a linearly increasing branch. The greatest differentiation between species is observed in the plateau branch. No significant species differences are observed in the early steeply rising branch and although the late, linearly increasing branch is not well defined for all species, there are indications that species differences are less in this branch than in the plateau region.

Although these generic resemblances are beyond question, the cumulant curves differ in detail in enough respects to permit the conclusion that there are several factors in radiosensitivity that vary independently between species.

4.4 *The early, steeply rising branch.*—The full extent of the early branch is shown in the inset in text-figure 1. This branch is a straight line (segment A–A) with a slope of 1.74, that extends from about 0.02 days to 20 days and perhaps beyond, covering a range of C_L values from 10^{-7} to more than 10^{-2} $(r/day)^{-1}$.

The cumulant curves of all species have this branch as a common asymptote. The agreement between species is remarkable, for no significant differences can be observed, either in slope or intercept. The species differ markedly in the time at which they break away from the early branch. The rabbit falls abruptly away from the common trend before the 10th day, mouse and rat fall off together and gradually between the 10th and 20th days; dog, guinea pig, and burro between the 20th and 30th days.

The values between 0.01 and 0.1 days were determined by observing the survival times in X- or *gamma*-ray beams at intensities of hundreds or



TEXT-FIGURE 1.—Cumulant lethality for 7 species of mammals, plotted against mean survival time on a log-log grid.

thousands of r per minute. It should be noted that these data come from several independent investigations (*9*, *13*, *14*).

The approximate square-law relation between dose rate and survival time was first described for survival of the mouse and rat (*1*), where, for survival times from 4 to 8 days, the data fitted the formula

$$It^2 \cong 16{,}000 \text{ roentgen days} \qquad [16].$$

The constants for the early branch in text-figure 1 are estimated to be

$$\log_{10} C_L = 1.74 \log_{10} t^* - 4.00 \qquad [17]$$

or

$$C_L = 10^{-4}(t^*)^{1.74} \qquad [18].$$

The interpretation given previously (*1*) for the approximate square-law behavior at early times was that, at times so short that recovery has not yet set in, the impulse injury function $\phi(t)$ rises approximately as the first power of time after irradiation

$$\phi(t) \propto t \qquad [19].$$

The cumulant function would then be

$$\int_0^t \phi(t)\, dt \; \alpha \frac{t^2}{2} \qquad [20].$$

It was indeed found in the earlier study that the relation between single dose (D) and mean survival time for supralethal doses obeyed the relation

$$Dt^* = \text{constant} \qquad [21]$$

as is required by the hypothesis that initially the single-dose injury function increases in proportion to time. Beyond this point inconsistency arises that has not yet been resolved (*1*). The breakdown is not a result of neglecting the stochastic element in the death process, since an analysis taking this into account reveals the same discrepancy.[6] By elimination, it becomes more likely that nonlinearity in the kinematics of radiation injury is the cause, and this is borne out by the discontinuity in the relation between size of single dose and survival time that is seen at about 4 days (*28*). Investigation of the time range from 0.1 to 5 days with continuous exposures is needed to resolve the dilemma.

It is reasonably certain that the early branch exhibits the time course of accumulation of radiation injury almost uninfluenced by recovery. The power law of accumulation and the absence of species differences are evidence of this.

The invariance of survival time between species at sufficiently high rates that has been noted for 6 mammalian species unexpectedly gives testimony in favor of one of the fundamental hypotheses of the theory of ethality. This hypothesis asserts that the value of the lethal bound of njury, M, is invariant with respect to species, etc. The most parsimonious explanation for the species in variance of the cumulant function at very early times is that: (*a*) the primary molecular damage produced by radiations must be of the same kind and degree in all species; (*b*) the subsequent chain reaction of injury must have essentially the same paths and time course, at least in Mammalia and at constant temperature, etc.; and (*c*) the end point of the process, as measured by death, must be quantitatively the same among mammals. This end point is the lethal bound, as defined.

This argument is compelling with regard to the modes of death that prevail in the first few days, but it serves at best to increase the plausibility of the hypothesis with regard to later times. Direct support for the hypothesis under any other conditions will be difficult to come by, because the intervention of recovery mechanisms, which are beyond question species-specific, place almost insuperable difficulties in the way of quantitative verification.

4.5 *The onset of recovery; species differences; correlations with single dose DL_{50} and with single dose mean survival time.*—The falling away from the early power function trend is a consequence of recovery processes. This

[6] Unpublished data.

follows from the proof, in the preceding paragraph, that the initial stages, at least, of the injury process are quantitatively identical for all mammals examined.

It is not possible to give a single measure of the recoverability of the various species because it is obvious from the shapes of the different curves that there are several systems involved that have independent recovery constants. We can neglect these complications and draw some semiquantitative correlations.

The numerical rank of the 7 species examined here with respect to various measures of radiation sensitivity, is given in table 2. It may be noted that:

1) Rank orders are correlated for all measures of acute sensitivity, whether single dose or daily dose.

2) C_L values in the 11- to 17-day period and reciprocal LD_{50}'s not only are correlated but also have almost constant ratio one to another for 6 species. The rank order is fairly well preserved at 200 to 300 days but the constancy of ratios is not. A recovery time deduced from single-dose-survival data is also in constant ratio with reciprocal LD_{50} and 11- to 17-day C_L.

3) The mean survival time of decedents following less than LD_{50} dosages is negatively correlated with LD_{50}. This is in agreement with the forms of the cumulant functions of sensitive and resistant species because, if physical sensitivity is constant and the recovery time is increased, the maximum intensity of injury following a single dose also increases, and the maximum is reached at a later time.

In summary, the properties of the C_L functions of the various species are highly correlated with other measures of the radiation response in the acute time period.

4.6 *The plateau branch.*—The most striking feature of this branch is the great spread between the most sensitive and the least sensitive species.

TABLE 2.—*Tabulation of some measures of radiation sensitivity derived from single-dose and duration-of-life lethality studies*

Species	LD_{50} (*r*)	Reciprocal LD_{50} $(r)^{-1}$	MST* (*days*)	Cumulant value 11–19 days† $(r/day)^{-1}$	Recovery constant (*days*)‡	Cumulant value— 200 days $(r/day)^{-1}$
Rabbit (*29*)	800	1.25×10^{-3}	11	4.5×10^{-3}	5	2.9×10^{-2}
Rat (*18*)	600	1.67×10^{-3}	12	8×10^{-3}	7	2.5×10^{-2}
Mouse (Average)	600	1.67×10^{-3}	12	8×10^{-3}	7	3.7×10^{-2}
Monkey (*30*)	450	2.2×10^{-3}	16	— — —	9	52×10^{-2}
Dog (*30*) (*31*)	325	3.1×10^{-3}	17	13×10^{-3}	12	10.5×10^{-2}
Burro (*26*)	§750	1.33×10^{-3}		13×10^{-3}	— — —	— — —
Guinea pig (*32*)	200	5×10^{-3}	17	13×10^{-3}	— — —	19×10^{-3}

*Mean survival time for less than LD_{50} dosages.
†Estimated value at single-dose MST of respective species.
‡Estimated from single-dose survival times (*33*).
§Co^{60} *gamma*.

At 200 days the guinea pig harbors about 10 times more radiation injury than the rat per unit dose rate (table 2).

The physiologic significance of the plateau period is that all, or the greater part of, the recovery processes of the organism have, by this time, come into a quasi-steady state with the injury that is continually being produced by the constantly impinging ionizing radiations. The plateau is not well attained until a time has elapsed that is at least 3 times the longest mean recovery time [7] of the system.

When we examine the C_L functions of guinea pig, dog, and rabbit we see that the final plateau value is not attained smoothly, but rather by two or more steps. This is evidence that at least that number of physiologic systems have recovery times spaced widely enough to permit such differentiation.

The times for guinea pig, dog, and rabbit, respectively, to attain their final plateaus are about 140, 140 and 100 days. We infer therefore that systems must be present having recovery times ranging between 50 and 30 days. The similarity of these times to the life expectation of the erythrocyte in these animals is too striking to be ignored. There is also the observation that deaths in the 60- to 200-day period are due to a characteristic syndrome of aplastic anemia (*10*). Mouse and rat do not show stepwise recovery in text-figure 1, but this is due to the obscuring effect of plotting together data from a number of strains. A thorough study of a homogeneous mouse population (*1*) reveals the same stepwise character, but the steps are not so distinct and the final plateau is fully attained in about 60 days.

The times required to reach the first plateau after breaking away from the early rising branch are in agreement with the mean recovery times deduced from single-dose lethality studies. The mean recovery times are listed in table 2.

When the final plateau is attained, recoverable injury no longer accumulates progressively. However, the component of nonrecoverable injury is accumulating continuously and the time finally arrives when this component exceeds in magnitude the recoverable injury. It is at this time that the final increasing branch of the cumulant function becomes recognizable. In some species the nonrecovering component becomes manifest much earlier than the control mean survival time. Mouse and rabbit are examples of this. In the guinea pig, on the other hand, the plateau branch is so predominant that the nonrecovering branch never becomes evident. It would appear, from examination of pathologic data on guinea pig and rabbit, that the different physiologic status of these species is reflected in differences in incidence of pathologic conditions (*10*).

Finally, there is the question whether the very existence of the broad final plateau might not be an artifact due to the introduction of the correction for natural aging. Since the long-lived species get much smaller corrections, and yet show plateaus as well defined as those for the mouse and rat, it does not seem probable that the plateau is an artifact. The

[7] The mean recovery time will be here defined as the reciprocal of the recovery rate constant.

slope of the plateau branch might well be affected by inaccuracy in the correction and for this reason the slopes are not compared.

4.7 *The late ascending (chronic) branch.*—This branch is perhaps the most important for society, but unfortunately it is the least well established. Some characteristics of this branch may be listed.

1) In a species for which the late branch is fairly well established, *e.g.*, mouse, both for X and *gamma* rays and for neutrons, the trend, on the log-log plot, is linear with unit slope. In other words, late injury accumulates in proportion to accumulated dose, or to time in the case of exposure at a constant rate. Suppose that C_L [Eq. 15] is increasing in proportion to time,

$$C_L=\frac{1}{I}\left(1-\frac{t^*}{t_0}\right)=kt \qquad [22]$$

so that

$$k=\frac{1}{I}\left(\frac{1}{t^*}-\frac{1}{t_0}\right) \qquad [23].$$

Equation [23] has long been used to fit survival data at low dose rates (*1*, *17*).

Increase of C_L as the first power of time is the behavior to be expected if injury arising from low levels of radiation cumulate with the natural aging process, accelerating pathologic tendencies but introducing no qualitatively new pathology. From what little is known, this appears to be the case. In contrast to the situation in the plateau period, we should expect to find in this final period no displacements of the physiologic steady states of greater magnitude than those that accompany aging. This expectation is on the whole confirmed by the available histologic and hematologic data on animals exposed at low levels.

2) There is a slight indication that species differences decrease when the late branch is fully attained. One notes that the dog and guinea pig have appreciably reduced their disadvantage over the mouse by 1,000 days, while the rat, on the other hand apparently lost its advantage over the mouse at 500 days. This behavior of the rat, like the anomalous response of the monkey, is open to another interpretation (see Sec. 4.10).

Tentative estimates of the dosage sensitivity constants for mouse, dog, and rabbit exposed to X or *gamma* rays, but expressed in terms of X rays, are

$$k_m\simeq 17\times 10^{-5}\,r^{-1} \quad \text{(mouse)} \qquad [24]$$

$$k_d\simeq 35\times 10^{-5}\,r^{-1} \quad \text{(dog)} \qquad [25]$$

$$k_r\simeq 9\times 10^{-5}\,r^{-1} \quad \text{(rabbit)} \qquad [26].$$

These values are in the rank order of reciprocal LD_{50}'s for these species (table 2). They may be inserted in equation [23] to estimate the reduction in life expectation by dose rate I within the domain of the late rising branch. The tentative nature of these estimates must be emphasized,

for the trend is barely established and then only for mortality from all causes. Accurate determination of dosage sensitivity for specific causes of death remains to be done.

3) The late branch of the cumulant functions of the experimental animals shows no recovery within the life spans of these animals. It should not be inferred from this that no recovery is possible at later times in the comparable component of the longer-lived species. For the present, however, the safest and most reasonable working assumption is that the chronic branch is nonrecovering in all species in the absence of medical intervention.

4.8 *RBE for gamma rays and the effect of protraction.*—The cumulant values for the *gamma*-ray data for mice and rats could be made to superimpose reasonably well on those for X rays by assuming a *gamma*-ray *RBE* of 0.5 (see Sec. 1 above). This factor is a correction both for radiation quality and for protraction, since all *gamma*-ray exposures to mice and rats were given over periods of 8 or 24 hours per day. It appears then that X and *gamma* rays have about the same relative effectiveness from the acute through the chronic range, and it is therefore probable that their mechanisms of action are very similar.

All except one of the guinea pig cumulant values are based on *gamma*-ray exposures and the same *RBE* was employed with the justification that it was satisfactory for mouse and rat. There is, however, no direct evidence that the guinea-pig-cumulant values for X-ray exposure would be as indicated in text-figure 1. Studies of guinea-pig survival under daily exposure to X rays in the dose-rate range from 10 r per day to 1 r per day are needed.

The data for the burro were obtained from *gamma*-ray exposures at a dose rate of about 50 r per hour, so that the exposure times range from 8 hours down to 0.5 hours. The effectiveness of *gamma* rays varies about 20 percent over this range of protractions (*34*) so that a small bias (probably below the level of significance) exists between the highest and the lowest daily dose results. The assumed *RBE* of 0.5 puts the burro data precisely on the early rising trend line, which is determined principally by X-ray values. It would appear therefore that this assumed value is also a reasonably good estimate of the *RBE* of protracted *gamma* rays in the case of the burro.

It is clear that the *RBE* of *gamma* rays and the effects of protraction need further investigation, because the few studies that have been done provide too small a base for the tremendous amount of work that is being done with these two radiation qualities, especially when one realizes, as is shown by this survey, that results with these two qualities are completely interconvertible.

4.9 *Comparison of X rays and fast neutrons.*—Neutron dosages were converted to rem units, based on the ratio of X ray to neutron LD_{50}'s (see Sec. 4.1). When the C_L for neutron-irradiated mice is compared to that for X and *gamma* rays, an interesting pattern appears. The *RBE* of neutrons (as rem) is effectively unity for 80 days, at which time the C_L

curve for neutron branches off from the X-ray curve and starts into the late rising trend. The coefficients of the late branch for neutrons and X rays, respectively, are

$$k_{mn} \simeq 34 \times 10^{-5} \text{ (rem)}^{-1} \quad [27]$$

$$k_{mx} \simeq 17 \times 10^{-5} \text{ r}^{-1} \quad [28].$$

The effectiveness of neutrons is double that of X rays in the late branch. It would appear therefore that when neutrons are equated to X rays by the LD_{50} response, this equality holds for all components of recoverable injury, but the nonrecovering component is twice as sensitive to neutrons. That late effects of daily exposure to neutrons have a greater *RBE* has been a matter of interest since it was originally reported (*5*, *7*). This analysis shows that the increase in *RBE* is not achieved by a gradual transition, but rather by the characteristically higher sensitivity of one response component; namely, the late rising or chronic branch. This finding serves better than any other to indicate that the recovering and nonrecovering injury components are different in kind.

4.10 *Monkey and rat; possible effect of intercurrent disease.*—The highest cumulant values observed for any species are those for the monkey (*17*).[8] Furthermore, there is in these data no evidence that the monkey cumulant is approaching a plateau as late as 250 days.

These observations are most disquieting, but there are other factors to take into account. Tuberculosis was enzoötic among these animals and the death rate from this cause was high (*17*). Also, analysis of the survival data shows that in all the treatment groups, including the 0.1 r per day level, the dying out was exponential from the beginning of treatment. Thus the mode of action of radiations must have been entirely different here than in other species, which show an entirely different sigmoid order of death.

These observations taken together suggest the possibility that when morbidity from other injury or disease processes reaches a high level, the radiation lethality process is radically altered, both quantitatively and qualitatively. In view of the fact that the great bulk of results to date are obtained from young, healthy populations as the starting point, these findings emphasize the need for information about the age-dependence of radiation injury and about the interaction with other kinds of debility.

The behavior of the rat cumulant function is perhaps an example of a similar situation, for the cumulant rises abruptly after 400 days (E–E, text-fig. 1). This is the age at which respiratory disease becomes enzoötic or epizoötic in some colonies and strains of rats; a similar pattern is noted in reports from widely separated laboratories working with various strains of rats. The relatively great sensitivity of the guinea pig in the acute and subacute period perhaps has a similar origin.

[8] In obtaining the cumulant values used here, deaths in the first 10 days of treatment were excluded from the averages and the 0.1 r per day level was taken as the control group.

The low slope of the plateau branch of the rat cumulant curve could be due to the effect of rat pneumonia on the survival of control rats. These show heavy mortality from this cause, starting after one year of age, and the expectation of life is greatly reduced thereby. Since this cause of death does not operate before 400 days, the life expectation for controls to be inserted into equation [15] should be the expectation that would prevail if this cause of death were removed. However, it should be made clear that even if control expectation for rats were assumed to be 1,000 days, which is considerably in excess of any ever observed (*35*), the plateau of the rat cumulant curve would not be moved into superposition on the mouse curve. Therefore there is a real difference between the mouse and rat in the late part of their cumulant curves.

5. Discussion

5.1 *Summary of preceding sections.*—An analysis of radiation lethality in mammals has been developed on the basis that: 1) many or all aspects of physiologic function are affected by radiations; 2) the responses of physiologic systems to irradiation, and the interactions between systems, are linear; 3) a lethal process arises which is a weighted sum of the injuries to the constituent systems; 4) death occurs when this process intersects with a lethal bound of injury; and 5) when aging is a significant factor, the effects of the aging process and the radiation process combine additively. The further approximation is made that the behavior of a population is described by a representative animal, taken to be the mean or median animal. This latter approximation permits the interpretation of data from small groups of animals, which is the burden of the present paper.

The data analyzed were mean survival times at various daily dosages. Data for 7 species were available. By using the above hypotheses, it is possible to deduce for each species a cumulant lethality function that describes the course physiologic injury would follow consequent to continous exposure if the species behaved according to hypothesis. No internal test of consistency is possible, but one can determine whether the derived lethality functions are consistent with other kinds of information. At least semiquantitative consistency is obtained for several types of comparison. The results obtained thus far justify the conclusion that the essential assumptions are realistic and that the generalizations that may be necessary for a more exact treatment will not make the problem intractable.

The most sensitive and the least sensitive species investigated differ by a factor of 10 in the plateau (steady state) values of their cumulant functions, and there is about a factor of 4 between the LD_{50} values of the same species.

The form of the cumulant function provides a basis for definitions of acute, subacute and chronic injury that would be consistent between species. For example, chronic injury in a given species might be defined as the injury manifested when the final branch, on which accumulated

injury is proportional to accumulated dose, becomes predominant over the plateau branch of equilibrated recoverable injury.

There is evidence that the chronic branch of the cumulant function shows less species variation than the horizontal branch. It will be a matter of considerable difficulty to assay this, for we have the experimental difficulty of investigating chronic survival, and the further problem of species differences in aging pattern.

The problems of the relative biological effectiveness of different radiation qualities and of different protractions are clarified insofar as it is possible to attribute the altered effectiveness to a specific phase of the lethal process. Thus, the increased relative effectiveness of neutron exposure at low daily dose over that at high daily dose is attributable to a specifically increased effectiveness of neutrons on the chronic, nonrecovering, injury component. On the other hand the relative effectiveness of X rays and *gamma* rays seems to be constant at all dose rates.

A problem in need of further investigation is that of the interactions between a radiation-induced lethal process and coexisting lethal processes of other kinds, especially those arising from disease. The case is of practical importance, and also affords one means of approaching the crucial question of how injury processes interact to determine lethality.

5.2 *Implications for further developments in the analysis of radiation lethality.*—The duration-of-life exposure condition has performed a valuable role in making it possible to shape, in broad outline, the complete spectrum of physiologic injury and lethality. No other experimental procedure could have served this purpose as well. Now that this perspective has been achieved, consideration should be given to the further steps needed to develop a powerful and accurate predictive calculus.

It is important that the gaps and uncertainties in our present knowledge of the cumulant functions be filled in. The most important step, however, is to put the analysis of radiation lethality on a system-specific basis. This suggests two experimental problems. The first is to carry out a program of differential irradiation of organs, tissues or other natural body divisions. One possible way of doing this is to use a group of diversely metabolized radioisotopes. The fact that these materials expose the animal to a time-dependent radiation intensity presents no additional problem, since the theory developed here is applicable in such circumstances (*2*).

The second problem is that of measuring the effects of radiations on a number of physiologic systems during the course of exposure, with concomitant measurement of the rates of mortality due to specific causes.

There is a possibility that with this information at least a first-order analysis of lethality can be made that deals quantitatively with the salient features of the problem. These may be listed as: 1) the general law of relation between injury and lethality; 2) the parameters of this relation for each physiologic system; 3) the mode of combination of different kinds of injury to produce a resultant lethality; 4) the radiation sensitivity and kinematics of the individual systems.

The objectives listed are, at least implicitly, the objectives of a great deal of the work now being done in the fields of physiology, histology, cytology, pathology, etc. The important point to be made is that there must be explicit recognition of the necessity for concerted effort. The tremendous advances in nuclear physics and engineering could never have emerged from the uncoordinated efforts of individual investigators, however brilliant some of the contributions may have been. In an even more imperative sense, the achievement of a rational biology of the integrated organism, especially as it bears on the problem of the stability of living systems, will require a long period of conscious coordination of research effort that must extend beyond the boundaries of individual laboratories and individual disciplines.

Bibliography

(*1*) Sacher, G. A.: The survival of mice under duration-of-life exposure to X rays at various dose rates. CH-3900,[9] 1950.

(*2*) ———: The lethality function for exponentially decaying exposure: Application to the toxicity of tritium. Quarterly Report, Division of Biological and Medical Research, ANL-5288, 1954, pp. 16–19.

(*3*) Brues, A. M., and Sacher, G. A.: Analysis of mammalian radiation injury and lethality. *In* Symposium on Radiobiology (Nickson, J. J., ed.). New York, Wiley, 1952, pp. 441–465.

(*4*) Lansing, A. T., ed.: Cowdry's Problems of Ageing, 3rd ed. Baltimore, Williams and Wilkins, 1952.

(*5*) Evans, T. C.: Effects of small daily doses of fast neutrons on mice. Radiology 50: 811–834, 1948.

(*6*) Henshaw, P. S.: Experimental roentgen injury. IV. Effects of repeated small doses of X-rays on blood picture, tissue morphology, and life span in mice. J. Nat. Cancer Inst. 4: 513–522, 1944.

(*7*) Henshaw, P. S., Riley, E. F., and Stapleton, G. E.: The biologic effect of pile radiations. Radiology 49: 349–360, 1947.

(*8*) Henshaw, P. S., Snider, R. S., Riley, E. F., Stapleton, G. E., and Zirkle, R. E.: Comparative late effects of periodic doses of fission neutrons and of gamma rays. MonH–115,[9] 1946.

(*9*) ———: Comparative late effects of single doses of fission neutrons and gamma rays. MonH-116,[9] 1946.

(*10*) Lorenz, E., Jacobson, L. O., Heston, W. E., Shimkin, M., Eschenbrenner, A. B., Deringer, M. K., Doniger, J., and Schweisthal, R.: Effects of long-continued total-body gamma irradiation on mice, guinea pigs and rabbits. III. Effects on life span, weight, blood picture, and carcinogenesis, and the role of the intensity of radiation. *In* Biological Effects of External X and Gamma Radiation (Zirkle, R. E., ed.). Part I. New York, McGraw-Hill, 1954, pp. 24–148.

(*11*) Lorenz, E.: Personal communication.

(*12*) Quastler, H., and Lanzl, E. F.: Biological evaluation of 20 million volt roentgen rays. IV. Efficiency and dosage range. Am. J. Radiol. 63: 566–574, 1950.

(*13*) Rugh, R., and Clugston, H.: The time-intensity relations of whole-body acute X-irradiation and protection by β-mercaptoethylamine. Radiation Res. 1: 437–447, 1954.

(*14*) Smith, D. E.: Personal communication.

[9] Information regarding the availability of these reports may be obtained by addressing requests to the Office of Technical Services, Dept. of Commerce, Washington, D. C.

(*15*) GRAHN, D.: Personal communication, 1954.

(*16*) THOMSON, J. F., TOURTELLOTTE, W. W., CARTTAR, M. S., COX, R. S., JR., and WILSON, J. E.: Studies on the effects of continuous exposure of animals to gamma radiation from cobalt 60 plane sources. Am. J. Roentgenol. 69: 830–838, 1953.

(*17*) BOCHE, R. D.: Effects of chronic exposure to X radiation on growth and survival. *In* Biological Effects of External Radiation (Blair, H. A., ed.). New York, McGraw-Hill, 1954, pp. 222–252.

(*18*) HAGEN, C. W., and SIMMONS, E. L.: Effects of total-body X-irradiation on rats. I. Lethal action of single, paired and periodic doses. MDDC-1210, 1947.[9]

(*19*) SACHER, G. A.: Daily dose lethality data for rabbits. Quarterly Report Division of Biological and Medical Research, ANL–4531, 1950, pp. 128–135.

(*20*) T'ANG, Y.: Les bases expérimentales de la radiothérapie totale et subtotale. J. belge-radiologie 28: 207, 1939.

(*21*) DUCUING, J., MARQUES, P., and MILETZKY, O.: Radiothérapie totale dans les maladies des organes hématopoïétiques. Modifications sanguinés et déductions pratiques. J. radiol. et électrol. 1: 250–257, 1937.

(*22*) DUCUING, J., MILETZKY, O., and MARQUES, P.: Recherches experimentales sur les modifications sanguinés provoqueés par des irradiations totales a l'aide des rayons X. Sang 11: 483, 1937.

(*23*) PROSSER, C. L., and MOORE, M. C.: The clinical physiology of dogs exposed to daily total-body doses of X rays. CH-3658,[9] 1947.

(*24*) SACHER, G. A.: Unpublished.

(*25*) RUST, J. H., TRUM, B. F., HEGLIN, J., MCCULLOH, E., and HALEY, T. J.: Effect of 200 roentgens fractional whole body irradiation in the burro. Proc. Soc. Exper. Biol. & Med. 85: 258–261, 1954.

(*26*) RUST, J. H., TRUM, B. F., WILDING, J. L., SIMONS, C. S., and COMAR, C. L.: Lethal dose studies with burros and swine exposed to whole body cobalt-60 irradiation. Radiology 62: 569–574, 1954.

(*27*) RUST, J. H.: Personal communication.

(*28*) QUASTLER, H.: Studies on roentgen death in mice. I. Survival time and dosage. Am. J. Roentgenol 54: 449–456, 1945.

(*29*) HAGEN, C. W., and SACHER, G. A.: Effects of X rays on rabbits. I. Mortality after single and paired doses. *In* Biological Effects of External X and gamma radiation (Zirkle, R. E., ed.). New York, McGraw-Hill, 1954, pp. 243–264.

(*30*) BOCHE, R. D., and BISHOP, F. W.: Studies on the effects of massive doses of X radiation on mortality in laboratory animals. *In* Biological Effects of External Radiation (Blair, H. A., ed.). New York, McGraw-Hill, 1954, pp. 3–10.

(*31*) PROSSER, C. L., PAINTER, E. E., and SWIFT, M. N.: The clinical physiology of dogs exposed to single total-body doses of X rays. MDDC–1272,[9] 1947.

(*32*) HAGEN, C. W., and SACHER, G. A.: The acute lethal action of single doses of X rays on guinea pigs. CH–3907,[9] 1951.

(*33*) SACHER, G. A.: Remarks on the relation between single LD_{50} and the mean survival times of decedents. Quarterly Report, Division of Biological and Medical Research, ANL-4571, 1951, pp. 106–114.

(*34*) THOMSON, J. F., and TOURTELLOTTE, W. W.: The effect of dose rate on the LD_{50} of mice exposed to gamma radiation from cobalt 60 sources. Am. J. Roentgenol. 69: 826–829, 1953.

(*35*) MCCAY, C. M.: Chemical aspects of ageing and the effect of diet upon ageing. *In* Cowdry's Problems of Ageing (Lansing, A. I., ed.), 3rd ed. Baltimore, Williams and Wilkins, 1952, pp. 139–202.

[9] Information regarding the availability of these reports may be obtained by addressing requests to the Office of Technical Services, Dept. of Commerce, Washington, D. C.

Level of Protein Intake and Nitrogen Excretion in Rats Following Total-Body X Irradiation[1]

JULIUS WHITE, BERNARD E. BURR,[2] HARRIETT T. COOL, PHILIP W. DAVID, *and* MONA S. ALLY,
National Cancer Institute,[3] Bethesda, Md.

The interest in recent years on the various aspects of the radiation syndrome has been indicated by the large number of publications and reviews. Some have dealt with the various approaches to explain the mechanisms of radiation protection (*1–4*). Recently an excellent review (*5*) appeared on the biochemical changes occurring in animals following X irradiation. Emphasis was placed on changes in nucleic acids, carbohydrates and fat metabolism, as well as in serum proteins. Reports concerning nitrogen metabolism have been quite limited.

Inasmuch as a major portion of nitrogen balance and excretion generally indicates protein synthesis and degradation, it represents an index to protein metabolism.

In a preliminary report (*6*), a marked increase in nitrogen excretion was observed in rats ingesting a relatively low protein diet and exposed to 450 r total-body X irradiation. This increase in nitrogen output was reflected chiefly in the urea fraction. This suggested an increase in protein catabolism. Gros and Mandel (*7*) reported an average 36-percent increase in nitrogen excretion and a 44-percent increase in allantoin excretion in rats established on a protein-free diet and subjected to 400 r. Gustafson and Koletsky (*8*) exposed male rats to 660 r, which resulted in a 30 percent greater excretion of nitrogen than pair-fed controls. Brues (*9*) has also found a body nitrogen loss that was reflected in the urea fraction of the urine in rats following exposure to 450 r.

In this paper we wish to report the effect of various concentrations of protein intake on the rate of nitrogen excretion in rats exposed to 450 to 500 r total-body X irradiation.

Materials and Methods

Male and female Sprague-Dawley[4] as well as Osborne-Mendel rats weighing 230 to 300 grams were used. Three diets were employed for

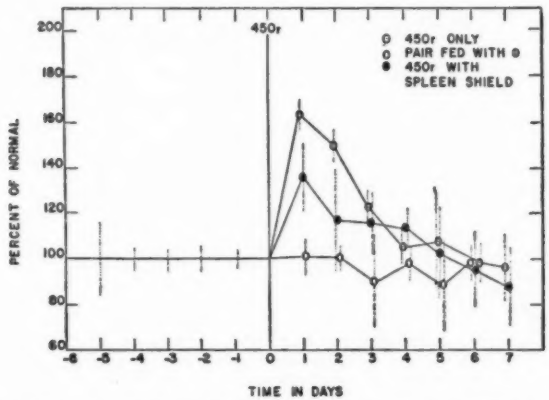
[1] Received for publication October 25, 1954.

[2] Part of this material has been presented to the faculty of Georgetown University by Bernard E. Burr in partial fulfillment of the requirements for the degree of Master of Science.

[3] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

[4] Hormone Assay Laboratories, Chicago, Ill.

this study: C-6, C-15 and C-20 respectively, previously described (*10*),[5] differing from each other only in the percent casein and starch present. C-6, C-15 and C-20 represent diets containing 6, 15 and 20 percent casein, respectively. The diets were isocaloric. In every case, the animals were allowed to ingest their respective diets for 30 days prior to any experimental procedure. They were housed individually in plastic cages with wire-mesh bottoms and so constructed that urine and feces could be collected separately. Urine and feces were collected every 24 hours and analyzed separately. Urine was analyzed for total nitrogen, urea nitrogen and ammonia nitrogen. Feces were analyzed for total nitrogen. Total



TEXT-FIGURE 1.—Average daily total nitrogen excretion in rats following whole-body X irradiation (C-6 diet). Preirradiation period of nitrogen excretion is fixed at 100. Vertical dotted lines indicate standard error. Curves are averages of 14 rats. Spleen-shielded curve is average of 5 animals.

nitrogen and ammonia nitrogen were determined by the micro-Kjeldahl method and urea was precipitated as the dixanthhydrol derivative. In the pair-feeding procedure, the pair-fed animal was given that amount of food consumed the previous day by the experimental animal.

The experimental animal received either 450 or 500 r total-body X irradiation.[6] Experimental data indicated that there was no significant difference in the nitrogen excretion in rats receiving 450 or 500 r.

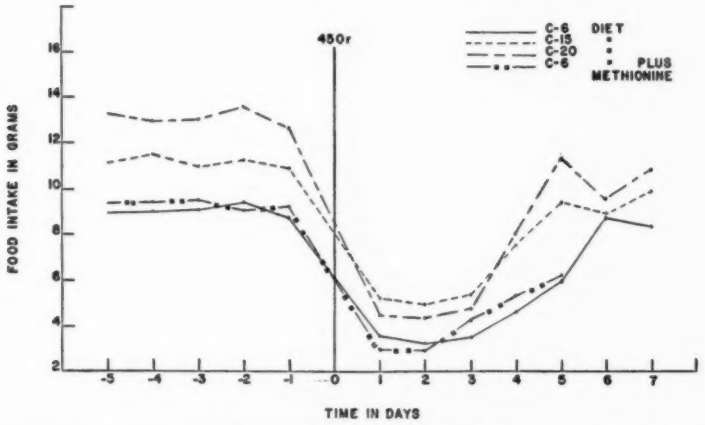
Results and Discussion

Animals ingesting the C-6 diet show a marked increase in nitrogen excretion (text-fig. 1) following exposure to whole-body X irradiation. Accompanying this increased nitrogen excretion there is a marked drop in food intake (text-fig. 2) during the first 1 to 4 days following X-ray treatment.

[5] Cod-liver oil was fed as a supplement twice weekly.

[6] The irradiation conditions were 186 KVP, 20 Ma., 0.25 mm. Cu + 0.55 mm. Al. Two tubes opposite each other were used to obtain a uniform tissue dose; their foci were 35 cm. from the center of the rats. The dosage rate was 205 r per minute.

No significant change in nitrogen excretion was seen in the pair-fed group. Since it is known (*11*) that exteriorizing and shielding the spleen protects animals against lethal doses of whole-body X irradiation, nitrogen excretion studies were made on such animals; an elevated nitrogen excretion resulted but it was significantly lower than the nonshielded group. This suggests that spleen protection against whole-body X irradiation not only involves the hematopoietic system but has a protective action against increased nitrogen metabolism resulting from radiation exposure. It can be noted that after the sixth day following



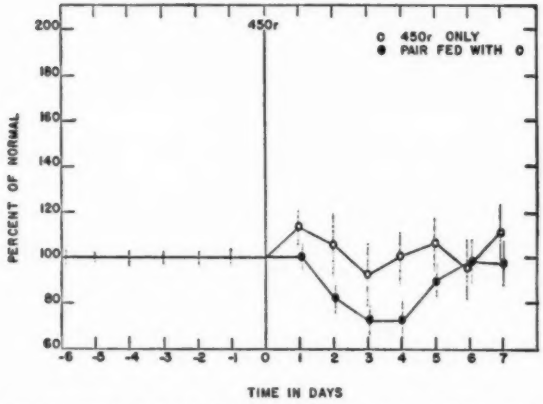
TEXT-FIGURE 2.—Average daily food intake of animals ingesting C-6, C-15, C-20 and C-6 plus methionine diets. Methionine (0.5 percent) was added and the cystine removed in the latter diet.

X irradiation there is an excretion of nitrogen equal to the preirradiation period.

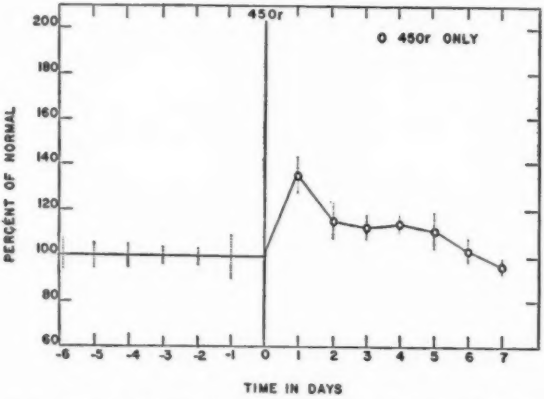
Animals ingesting the C-20 diet show an increased nitrogen excretion following X-ray treatment (text-fig. 3), but the increased excretion is considerably less than that of animals similarly treated in the C-6 diet (text-fig. 1). Pair-feeding experiments actually resulted in nitrogen retention in the pair-fed nonirradiated controls, suggesting that these pair-fed animals may be utilizing their stored fats as a source of energy and sparing nitrogen. A marked drop in food intake following irradiation was also observed (text-fig. 2) in this group.

Animals ingesting the C-15 diet show an increased nitrogen excretion (text-fig. 4) similar to that of the animals of the C-20 group, with the exception of the first day when the values are intermediate between the C-6 and C-20 group. No paired feeding was done in this group. Lowered food intake after X ray was also noted (text-fig. 2).

The diets employed (C-6, C-15 and C-20) in these experiments were somewhat deficient in their vitamin B_{12} content. The methionine content of the C-6 diet was lower than the C-15 and C-20 diets. However, the addition of methionine (0.5 percent) in place of the cystine to the C-6



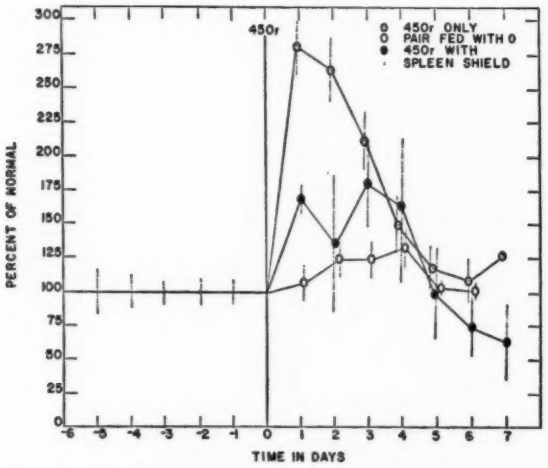
TEXT-FIGURE 3.—Average daily total nitrogen excretion in rats following whole-body X irradiation (C-20 diet). Preirradiation period of nitrogen excretion fixed at 100. Vertical dotted lines indicate standard error. Curves are averages of 12 rats.



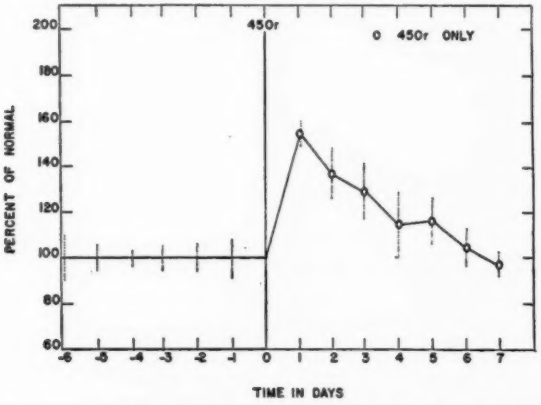
TEXT-FIGURE 4.—Average daily total nitrogen excretion in rats following whole-body X irradiation (C-15 diet). Preirradiation period of nitrogen excretion fixed at 100. Vertical dotted lines indicate standard error. Curve is average of 4 animals.

diet (text-fig. 2), which would have a sparing action on vitamin B_{12}, resulted in no greater food consumption or growth when fed to rats than the C-6 diet alone. Furthermore, irradiated rats ingesting the C-6 + methionine diet showed essentially the same nitrogen excretion as did the animals ingesting the C-6 diet alone (unpublished data).

The marked increased excretion of nitrogen of animals on the C-6 diet is reflected chiefly in the urea fraction of the urine (text-fig. 5). Since urea is the chief end product of protein metabolism, the elevated nitrogen excretion represents a measure of protein breakdown. Pair-fed animals did not show any marked increase in urinary urea, while the spleen-shielded animals showed an increase parallel to their total nitrogen excre-



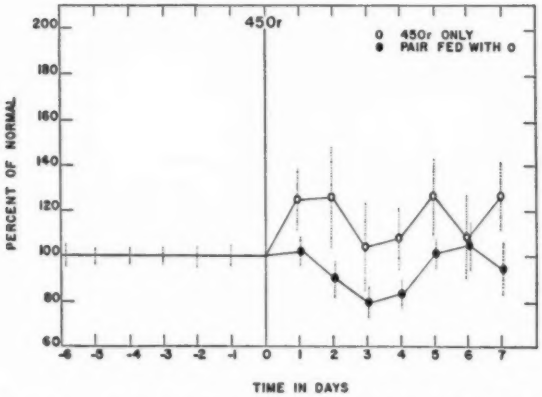
TEXT-FIGURE 5.—Average daily urea-nitrogen excretion in rats following whole-body X irradiation (C-6 diet). Preirradiation period of urea excretion fixed at 100. Vertical dotted lines indicate standard error.



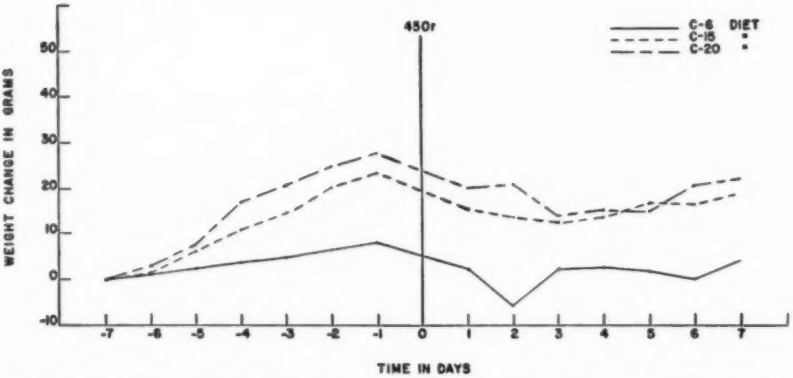
TEXT-FIGURE 6.—Average daily urea-nitrogen excretion in rats following whole-body X irradiation (C-15 diet). Preirradiation period of urea excretion fixed at 100. Vertical dotted lines indicate standard error.

tion. Animals ingesting the C-15 and the C-20 diets show similar increased levels of urea excretion (text-figs. 6 and 7).

It is not surprising that upon irradiation there results a marked increase in nitrogen excretion in rats ingesting the C-6 diet. Animals on this diet weighing 200 to 300 grams gained weight very slowly at a rate of 2 to 5 grams per week (text-fig. 8). Animals on the C-15 and C-20 diets gain at a rate of 15 to 18 and 21 to 26 grams per week, respectively. In text-figure 9 are shown the nitrogen balance curves of rats on the C-6, C-15 and C-20 diets, respectively. All 3 groups show a negative nitrogen balance

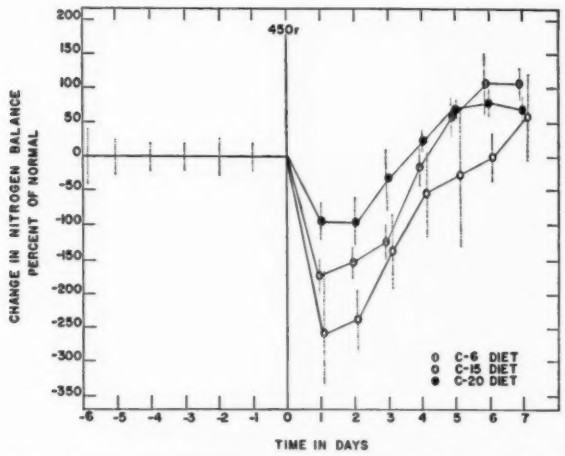


TEXT-FIGURE 7.—Average daily urea-nitrogen excretion in rats following whole-body X irradiation (C-20 diet). Preirradiation period of urea excretion fixed at 100. Vertical dotted lines indicate standard error.



TEXT-FIGURE 8.—Average daily weight changes of rats ingesting C-6, C-15 and C-20 diets, respectively.

following whole-body X irradiation, the C-20 group showing the greatest retention of nitrogen. The negative nitrogen balance of the C-15 group would be expected to be between the C-6 and the C-20 groups but actually is greater than the C-20 group. This, in part, is attributable to the large standard error of the C-15 group, particularly the first day. The C-15 curve represents only 4 animals. The curves for the C-6 and C-20 represent 14 and 12 animals, respectively, which were pair-fed. Equal numbers of males and females were used. A total of 30 C-6 and 24 C-20 animals were actually studied and the results are essentially identical with those of the 14 C-6 animals and of the 12 C-20 animals as presented in text-figure 9. The death incidence of rats in the C-6 and C-20 groups in the first 5 days is 20 and 15 percent, respectively. No early deaths are observed on the C-15 diet (unpublished data). After the first 3 to 4 days



TEXT-FIGURE 9.—Average daily nitrogen balance in rats following whole-body X irradiation. Preirradiation nitrogen balance set at a baseline of zero. Vertical dotted lines indicate standard error.

following X irradiation, there is a greater consumption of food as is shown in text-figure 2 accompanied by a gradual increase in weight. During the first few days following X irradiation there is a marked loss in weight, in some cases exceeding 30 grams. With the return to normal food intake, nitrogen is stored during the repletion period.

The experimental animals receiving the C-20 diet show a high total caloric intake, a positive protein caloric balance and a continued weight gain during the control period (table 1). After irradiation the total caloric intake is reduced, the protein caloric balance is negative and the animals lose weight for the first 4 days. This is followed by increase in the total caloric intake with associated positive protein caloric balance and weight gain.

The experimental animals on the C-15 diet show a positive protein caloric balance, a sustained weight gain and a high total caloric intake during the control period (table 2). Following irradiation there is reduced total caloric intake accompanied by a negative protein caloric balance and weight loss. After the fourth day of the experimental period total caloric intake increases and a positive protein caloric balance is seen associated with weight gain.

During the control period, the animals on the C-6 diet show a positive protein caloric balance and a sustained weight gain indicating a positive total caloric balance (table 3). Following irradiation these animals reflect the physiological injury with a substantial reduction in total caloric intake with concomitant weight loss and negative protein caloric balance. It should be noted that on the last days of the experimental period the protein caloric balance reverts to positive and the total caloric balance approaches control levels; however, the weight loss continues. No ex-

TABLE 1.—*Average daily caloric balance (12 animals) derived from proteins on the C-20 diet before and after total-body X irradiation*

Day	BW*	△BW†	T. cal.‡	Calories from protein			Un/Tn§
				Intake	Output	Balance	
1	261	.....	68. 74	10. 00	5. 24	+4. 76	0. 71
2	270	+9	66. 69	9. 70	5. 57	+4. 13	. 71
3	275	+5	67. 72	9. 85	5. 29	+4. 56	. 73
4	278	+3	69. 77	10. 15	4. 92	+5. 23	. 76
5	281	+3	65. 66	9. 55	4. 91	+4. 64	. 72
450 r total-body X irradiation							
6	271	−10	22. 57	3. 28	6. 05	−2. 77	0. 72
7	267	−4	22. 57	3. 28	5. 61	−2. 33	. 74
8	262	−5	24. 62	3. 58	4. 77	−1. 19	. 74
9	264	+2	42. 07	6. 12	5. 21	+0. 91	. 76
10	266	+2	58. 48	8. 51	5. 37	+3. 14	. 76
11	275	+9	50. 27	7. 31	5. 00	+2. 31	. 74
12	276	+1	56. 43	8. 21	5. 39	+2. 82	. 74

*Body weight.
†Daily weight change.
‡Total caloric intake.
§Ratio of urinary urea nitrogen to urinary total nitrogen.

TABLE 2.—*Average daily caloric balance (4 animals) derived from proteins on the C-15 diet and after total-body X irradiation*

Days	BW*	BW†	T. cal.‡	Calories from protein			Un/Tn§
				Intake	Output	Balance	
1	288	.....	57. 46	6. 18	3. 97	+2. 21	0. 71
2	291	+3. 0	59. 51	6. 40	4. 01	+2. 39	. 72
3	292	+1. 0	56. 43	6. 07	3. 82	+2. 25	. 74
4	294	+2. 0	57. 46	6. 18	4. 02	+2. 16	. 73
5	298	+4. 0	56. 43	6. 07	4. 06	+2. 01	. 74
450 r total-body X irradiation							
6	287	−11. 0	25. 65	2. 76	5. 72	−2. 96	0. 76
7	279	−8. 0	26. 68	2. 87	5. 07	−2. 20	. 76
8	276	−3. 0	28. 22	3. 03	4. 73	−1. 70	. 75
9	276	0. 0	39. 50	4. 25	4. 60	−0. 35	. 72
10	278	+2. 0	48. 22	5. 18	4. 48	+0. 70	. 74
11	280	+2. 0	47. 20	5. 07	4. 03	+1. 04	. 74
12	280	0. 0	51. 30	5. 51	3. 90	+1. 61	. 74

*Body weight.
†Daily weight change.
‡Total caloric intake.
§Ratio of urinary urea nitrogen to urinary total nitrogen.

planation for this observation can be offered at this time. It does suggest, however, that the metabolic recovery of the animals receiving a C-6 diet is different from that of the animals receiving isocaloric diets of higher protein content.

It is known that following irradiation the experimental animals show a reduced caloric intake. This demands that endogenous energy sources

TABLE 3.—*Average daily caloric balance (14 animals) derived from proteins on the C-6 diet before and after total-body X irradiation*

Days	BW*	BW†	T. cal.‡	Calories from protein			Un/Tn§
				Intake	Output	Balance	
1	220. 1	.....	47. 20	2. 56	1. 32	+1. 24	0. 44
2	221. 8	+1. 7	46. 17	2. 51	1. 23	+1. 28	. 45
3	223. 4	+1. 6	47. 20	2. 56	1. 29	+1. 27	. 47
4	224. 8	+1. 4	48. 22	2. 62	1. 25	+1. 37	. 47
5	226. 4	+1. 6	45. 14	2. 45	1. 16	+1. 29	. 46
450 r total-body X irradiation							
6	221. 3	−5. 1	17. 96	0. 97	2. 42	−1. 45	0. 58
7	214. 9	−6. 4	16. 93	0. 92	2. 18	−1. 26	. 59
8	207. 6	−7. 3	18. 47	1. 00	1. 75	−0. 75	. 61
9	214. 8	+7. 2	24. 11	1. 31	1. 46	−0. 15	. 52
10	214. 7	−0. 1	30. 78	1. 67	1. 26	+0. 41	. 48
11	213. 5	−1. 2	46. 17	2. 51	1. 19	+1. 32	. 48
12	211. 3	−2. 2	44. 12	2. 39	1. 12	+1. 27	. 49

*Body weight.
†Daily weight change.
‡Total caloric intake.
§Ratio of urinary urea nitrogen to urinary total nitrogen.

make up the energy deficit and should be manifest in a subsequent loss of weight. Our results are in agreement with this general observation. The experimental patterns which we observe—the reduced caloric intake, the negative protein caloric balance, and the weight loss—strongly indicate that one role of the nitrogenous compounds is protein catabolism to meet the energy deficit. The constancy of the urea-N/total N ratio in the control and experimental periods suggests that protein catabolism is unimpaired.

The negative protein caloric balance seen immediately after irradiation is not what one would expect in simple total calorie reduction. For example, there seems to be a lack of protein sparing on the first 2 days of the experimental period, perhaps suggesting that some other mechanism directly associated with the irradiation injury is being manifest in nitrogen excretion.

It is also possible that X irradiation may temporarily increase the metabolic requirements for one of the essential amino acids which may be limited in the C-6 diet. This would necessitate a greater catabolism of body substance to meet the immediate emergency than would be necessary with a 20-percent protein diet.

A part of this increased nitrogen excretion may be attributed to the direct injury effect of X irradiation.

Summary

Rats fed a low protein diet (6-percent casein) show a marked increase in nitrogen excretion, while those ingesting a 15- or 20-percent casein diet show a much lower increase in nitrogen excretion when given 450 to 500 r total-body X irradiation.

These increases in nitrogen excretion are to a great extent due to increased urea excretions and suggest increased protein catabolism.

Food intake in all 3 groups is markedly reduced during the first 3 or 4 days following X irradiation.

As a result of radiation exposure, loss of appetite ensues, and coupled with radiation effects, demands for energy from endogenous sources are increased.

Shielding the spleen in the C-6 group during X irradiation results in partial protection against increased nitrogen excretion following radiation exposure.

References

(*1*) PATT, H. M.: Some aspects of the biological action of high energy radiations. Ann. Rev. Nuclear Sc. 1: 495–524, 1952.

(*2*) ——— : Protective mechanisms in ionizing radiation injury. Physiol. Rev. 33: 35–76, 1953.

(*3*) HOLLAENDER, A., and STAPLETON, G. E.: Fundamental aspects of radiation protection from a microbiological point of view. Physiol. Rev. 33: 77–84, 1953.

(*4*) BRUES, A. M., and PATT, H. M.: Mechanisms of protection against mammalian radiation injury. Physiol. Rev. 33: 85–89, 1953.

(*5*) ORD, M. G., and STOCKEN, L. A.: Biochemical aspects of the radiation syndrome. Physiol. Rev. 33: 356–386, 1953.

(*6*) WHITE, J., and BURR, B. E.: Nitrogen excretion following whole-body X-irradiation. (Abstract.) Cancer Res. 12: 307–308, 1952.

(*7*) GROS, C., and MANDEL, P.: Variations in the minimal endogenous nitrogen consumption as a result of total X-ray irradiation. Compt. rend. Acad. Sc. 231: 631–633, 1950.

(*8*) GUSTAFSON, G. E., and KOLETSKY, S.: Nitrogen metabolism following whole body X-irradiation. Am. J. Physiol. 171: 319–324, 1952.

(*9*) BRUES, A. M.: Chronic external irradiation and neutron radiobiology. Nuclear Sc. Abstr. 7: 7, 1953.

(*10*) WHITE, J., COOL, H. T., STANDER, R. S., and DAVID, P. W.: Metabolism of N^{15}-labeled *p*-dimethylphenylenediamine in rats. J. Nat. Cancer Inst. 15: 529–537, 1954.

(*11*) JACOBSON, L.: Evidence for a humoral factor (or factors) concerned in recovery from radiation injury: *A review*. Cancer Res. 12: 315–325, 1952.

Cirrhosis of the Liver in Rats Following Total-Body X Irradiation[1,2]

JULIUS WHITE, CHARLES C. CONGDON, PHILIP W. DAVID, *and* MONA S. ALLY, *National Cancer Institute,*[3] *Bethesda, Md.*

In a previous communication (*1*) it was indicated that the nitrogen excretion following total-body X irradiation was higher in rats ingesting a 6-percent casein diet than in those ingesting a 20-percent casein diet. It was also indicated that the urea nitrogen/urinary nitrogen ratio was higher in the former group of rats. Four days following this radiation exposure both groups began to show a positive nitrogen balance, a positive protein caloric balance and, in the 20 percent protein-fed group, a positive weight change. However, the rats ingesting the 6-percent casein diet continued to show weight losses.

The failure of the rats ingesting the 6-percent casein diet to gain weight early in the postirradiation period led us to study the possible changes which might occur in some of the organs of these animals if they were allowed to continue on this diet for a prolonged period of time.

Materials and Methods

Male and female Sprague-Dawley[4] rats weighing 110 to 120 grams were divided into 4 groups and placed on 1 of the 4 diets indicated in table 1. Each animal was housed individually in a wire mesh-bottom suspended cage and was allowed to consume its respective diet *ad libitum*. The animals were allowed to ingest these diets for 30 days. They were then exposed to 450 or 500 r total-body irradiation[5] and were continued on their respective diets until they had died or were sacrificed when they began showing marked loss in weight and decreased food intake. At the time of death, gross autopsy examinations were carried out and pieces of liver were taken for subsequent microscopic study.

---

[1] Received for publication October 25, 1954.

[2] Presented in part at the meetings of the Radiation Research Society, Cleveland, Ohio, May, 1954.

[3] National Institutes of Health, Public Health Service, U. S. Department of Health, Education, and Welfare.

[4] Hormone Assay Laboratories, Chicago, Illinois.

[5] The irradiation conditions were 186 KVP, 20 Ma., 0.25 mm. Cu + 0.55 mm. Al. Two tubes opposite each other were used to obtain a uniform tissue dose; their foci were 35 cm. from the center of the mice. The dosage rate was 205 r per minute.

TABLE 1.—*Composition of diets*

	A.A.* (*gm.*)	C-6 (*gm.*)	C-15 (*gm.*)	C-20 (*gm.*)
Casein (crude)	0. 0	60	150	200
Sucrose	658. 5	150	150	150
Inorganic salts	40. 0	40	40	40
Starch	0. 0	475	385	335
Crisco	100. 0	240	240	240
Yeast (Brewer's)	0. 0	20	20	20
Cod-liver oil	0. 0	10†	10†	10†
Cystine	2. 0	5	5	5
DL-Methionine	8. 0			
DL-Threonine	10. 5			
L-Histidine	9. 5			
L-Tryptophane	4. 0			
Glycine	1. 0			
DL-Alanine	4. 0			
DL-Serine	2. 0			
L-Tyrosine	6. 0			
L-Proline	2. 0			
L-Hydroxyproline	1. 0			
L-Aspartic acid	2. 0			
L-Glutamic acid	20. 0			
L-Arginine	5. 0			
L-Lysine	15. 0			
L-Leucine	10. 0			
DL-Isoleucine	16. 0			
DL-Valine	20. 0			
DL-Phenylalanine	8. 0			
Sodium bicarbonate	13. 0			
Agar	20. 0			
Mazola oil	20. 0			
α-Tocopherol	0. 5			
Total	998. 0	1, 000	1, 000	1, 000

*Vitamin supplements in mg.: Thiamin 5, riboflavine 10, pyridoxine 5, calcium pantothenate 5, nicotenamide 10, p-aminobenzoic acid 300, inositol 1,000, folic acid 5, biotin 1, choline 1,000, and 2-methyl 1,4-naphthaquinone 2.
†Fed as a separate supplement.

TABLE 2.—*Cirrhosis of the liver following 450 r total-body X irradiation on various diets*

Diet	No. of animals	No. deaths in 70 days	Cirrhosis of liver after 145 days
C-6	45	17	15
C-15	46	5	0
C-20	45	19	0
A. A.	20	2	0
C-6 controls	50	0	0*

*No cirrhosis after 565 days.

Results

A total of 45 animals were placed on the C-6 diet (table 2). Of this group, 17 died within 70 days after irradiation. In the remaining 28 animals, 15 developed cirrhosis of the liver, became moribund or died between the 145th and 283rd day. Two of the remaining 13 animals

are still alive after 400 days. Eleven of the 13 animals died between the 119th and 288th day. Each of these showed gross evidence of pulmonary infection.

The distribution of cirrhosis was nearly equally divided between the males and females (7 and 8, respectively). Grossly, the livers of the rats with cirrhosis showed a variation from mild degrees of nodular atrophic cirrhosis to the advanced condition demonstrated in figure 1. The cirrhotic parenchyma was usually yellow in color. Histologically, the regenerated liver lobules were separated by bands of connective tissue filled, in some livers, with ceroid pigment-laden cells (figs. 2 and 3). Numerous small bile ducts were also present. The regenerated liver cord cells frequently contained a large vacuole filled with sudanophilic material (fig. 4). There was no evidence of tumor formation.

Forty-six rats were placed on the C-15 diet. Five animals died as a result of radiation exposure in the first 70 days. Between the 105th and 223rd day, 14 died of pulmonary infection. Laparotomies were performed on the remaining 27 animals on the 225th day and no gross evidence of cirrhosis was observed. Twenty-three of these animals died with pulmonary infection between 227 and 396 days. Four are still alive.

Forty-five animals were placed on the C-20 diet before and after irradiation. Nineteen died before the 70th day. Between the 76th and 223rd day, 17 died of pulmonary infection. There was no evidence of cirrhosis. Laparotomies were performed on the remaining 9 and there was no gross evidence of cirrhosis. Seven died between the 264th and 393rd day without evidence of cirrhosis. Two are still alive.

Twenty animals were placed on the A.A. diet (amino acid mixture equivalent to the amino acid content in 15-percent casein). Two of these animals died on the second day following irradiation and the remaining 18 animals were placed on the Purina chow diet on the 30th day following X irradiation. All 18 rats are still alive after 400 days.

Discussion

It has been generally assumed from morphologic studies that the liver is relatively resistant to X irradiation. Furth and Upton (*2*) have recently reviewed the effects of irradiation on the liver in various species. In general, doses greater than 12,000 r appear to be necessary to cause significant injury to the liver, although 2,500 to 12,000 r will cause damage to a lesser degree. Koletsky and Gustafson (*3*) have reported that after exposing the livers of rats by intravenous injection of radioactive gold equivalent to 40,000–60,000 r, enough liver cells remain undamaged to allow recovery although ultimately cirrhosis appears. Patt and Brues (*4*) in a review of the physiology of radiation injury have also reported that morphologically the liver appears to be relatively resistant. Since a number of chemical changes are reported to take place in the irradiated liver, they pointed out that metabolic disturbances in the liver are possible which are not evidenced by alterations in the morphology.

In this report the high incidence of cirrhosis of the liver in the group ingesting the low protein diet (C-6) and of the absence of cirrhosis in the other 3 groups of animals suggests a metabolic impairment resulting from the combination of total-body irradiation injury and the low protein diet. Low protein diets (4 percent) have produced cirrhosis of the liver (*5*). However, the 6-percent casein diet employed in part of these experiments does not produce cirrhosis by itself. In control animals used in this laboratory (nonirradiated on the C-6 diet) no case of cirrhosis has appeared even after 18 months on this regimen.

The choline content of the 3 protein diets was equal. This is probably not a factor in the cirrhosis production even though the C-20 and C-15 groups consumed more food. Whether exposure to radiation interferes with the utilization of cirrhosis-preventing components (choline, methionine, methyl groups, etc.) in animals ingesting a low protein diet is unknown at the present time.

Pathologically, the character of the cirrhosis resembles the ordinary type of cirrhosis produced in rats by deficiency states. This is in contrast to the postnecrotic type of cirrhosis seen in animals receiving large injections of radioactive colloidal gold.

Pulmonary infection was a prominent cause of death in many of these rats, presumably due in part to the increased susceptibility to infection following total-body irradiation.

Summary

Rats fed a low protein diet (6 percent casein) developed cirrhosis of the liver in 53 percent (15 out of 28) of the animals which survived 450 or 500 r total-body X irradiation.

Rats fed either a 15- or 20-percent casein diet, or a diet in which the 15-percent casein was replaced by an amino acid mixture for a 30-day period, did not show cirrhosis for periods as long as 400 days following 450 or 500 r total-body X irradiation.

Pulmonary infection was a prominent cause of death in most of the groups of irradiated rats.

References

(*1*) White, J., Burr, B. E., Cool, H. T., and David, P. W.: Level of protein intake and nitrogen excretion in rats following total-body X irradiation. J. Nat. Cancer Inst. 15: 1145–1154, 1955.

(*2*) Furth, J., and Upton, A. C.: Vertebrate radiobiology: Histopathology and carcinogenesis. Ann. Rev. Nuclear Sci. 3: 303–338, 1953.

(*3*) Koletsky, S., and Gustafson, G.: Liver damage in rats from radioactive colloidal gold. Lab. Invest. 1: 312–323, 1952.

(*4*) Patt, H. M., and Brues, A. M.: The pathological physiology of radiation injury in the mammal. II. Specific aspects of the physiology of radiation injury. *In* Radiation Biology. Vol. 1, Part 2, Chapt. 15. New York, McGraw-Hill, 1954, pp. 986–988.

(*5*) Patek, A. J., Plough, I. C., and Bevans, M.: Relative effects of protein and lipotropic substances in the treatment of nutritional cirrhosis in rats. Ann. New York Acad. Sc. 57: 772–775, 1954.

PLATES

PLATE 67

FIGURE 1.—Nodular atrophic cirrhosis of the liver of an irradiated rat on the C-6 low protein diet.

FIGURE 2.—Section through one lobe of a liver showing advanced nodular atrophic cirrhosis. Trichrome stain. × 20

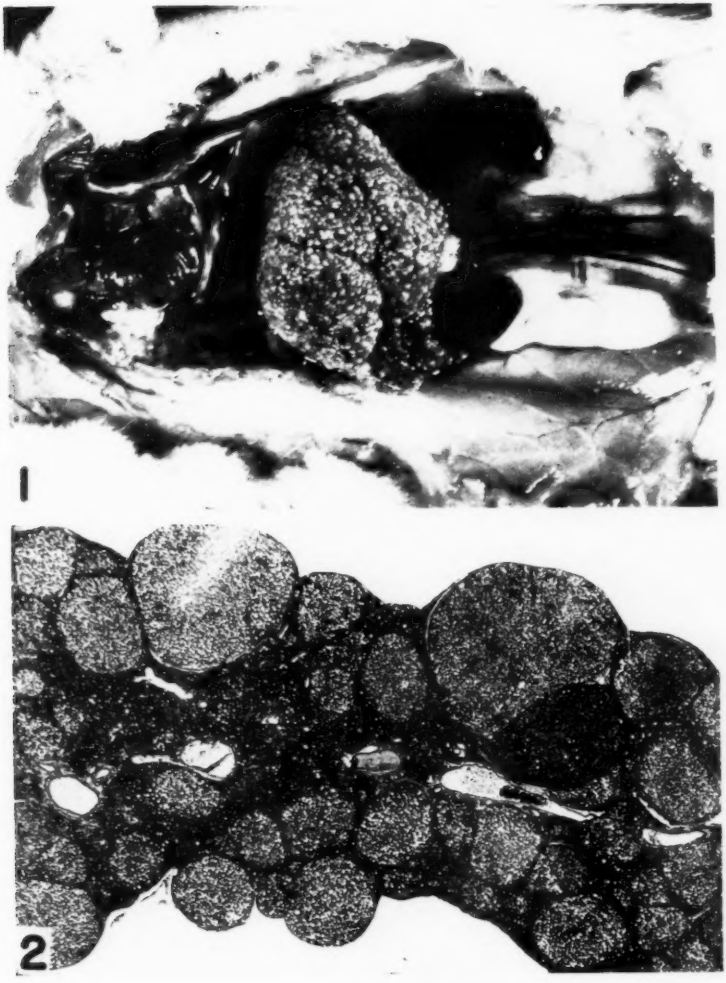
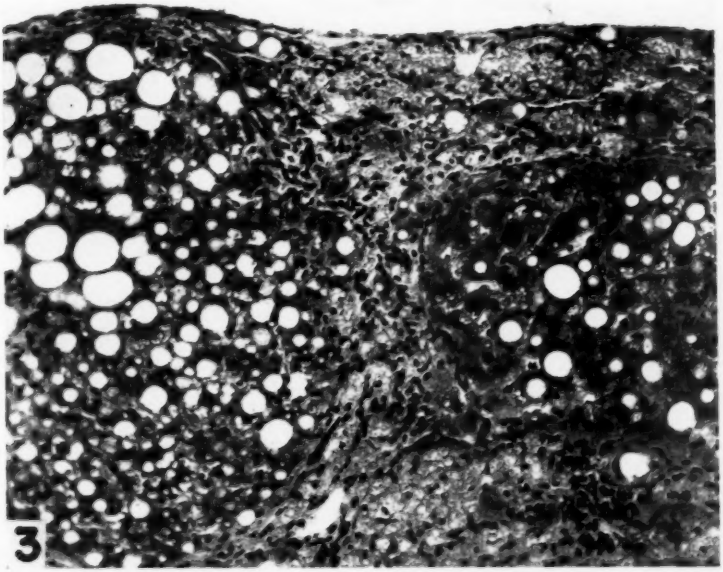
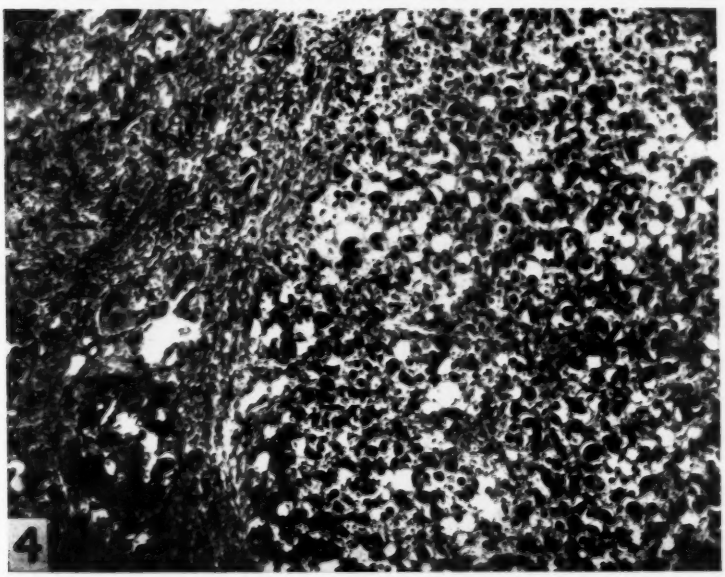

1
2

PLATE 68

FIGURE 3.—Regenerated liver lobules showing many vacuolated liver cells. The band of tissue between the lobules shows ceroid pigment-laden cells and connective tissue. Some of the elongated nuclei probably come from proliferating bile ducts. Hematoxylin and eosin. × 215

FIGURE 4.—Sudan stain on frozen section showing large fat droplets in parenchymal cells. × 105


3


4

The Journal of the National Cancer Institute is published bimonthly by the National Cancer Institute of the National Institutes of Health, Bethesda, Md. Six issues, beginning with number 1 in August, comprise each yearly volume. The Journal publishes original observations and investigations in laboratory and clinical research in cancer. Manuscripts are considered for publication with the understanding that they have not been published previously.

Publication is not limited to members of the National Cancer Institute or to those whose work is supported by Government funds. Manuscripts should be submitted to the Director, National Cancer Institute, Bethesda 14, Md.

Suggestions to Authors

1. Submit an original and two carbon copies of the manuscript. Follow the style of a recent copy of the Journal, especially for illustrations, references, and tables. Type all material double spaced.

2. In the by-line, include the name of the institution with which each author is connected. Official title or academic degree is optional.

3. Begin each page with a new paragraph; *i.e.*, each page should contain one or more complete paragraphs, and no paragraph should carry over from one page to the next unless it is longer than a full page. Do not begin a new paragraph on a page carrying a run-over paragraph. This is a requirement of the Government Printing Office.

4. Insert each footnote in the text immediately after the line in which it is mentioned.

5. Type each table on a separate page, to be the page immediately following the paragraph in which the table is first mentioned.

6. Graphs, charts, and drawings are printed in the text and referred to as text-figures. Legends for text-figures should be included in the text immediately following the paragraph in which the text-figure is first mentioned. A drawing three times the size of that desired for the final figure is preferable. The lettering should be uniform and of such size that in the reproduction it will be of the same size as that of the type used in the text. The original drawings reproduce best and are therefore preferable. If two additional copies (photostats, etc.) of each drawing are also submitted, review of the manuscript is expedited.

7. Photographs are printed together at the end of each article, as plate pages. The maximum size for each printed plate page is 5¼ by 8 inches, and the photographs should be combined into as few plates as possible. Photographs should be submitted either in the exact size, or exactly double the size, of the final printed reproduction. Mount the photographs for each plate together on one piece of heavy cardboard. Number each photograph consecutively. Type the legends for each plate on a separate page. If, in addition, two unmounted duplicate prints of each photograph are also submitted, review of the manuscript will be expedited.

Published by the U. S. Department of Health, Education, and Welfare in accordance with section 220, title 44, U. S. Code

The printing of this publication has been approved by the Director of the Bureau of the Budget on 18 August 1954

UNITED STATES GOVERNMENT PRINTING OFFICE, WASHINGTON : 1954

For sale by the Superintendent of Documents, U. S. Government Printing Office
Washington 25, D. C. - Price per copy varies with each issue.

Subscription price per year in the United States, Canada, and Mexico, $7.50; Foreign, $9.25

CONTENTS

JOURNAL OF THE NATIONAL CANCER INSTITUTE • VOL. 15 · NO. 4 · FEBRUARY 1955

Page

The histopathology of bacterial infection in irradiated mice. *Charles C. Congdon, Fletcher P. Williams, Jr., Robert T. Haberman,* and *Egon Lorenz*... 855

Regenerative growth in the urodele forelimb following ultraviolet radiation. *Elmer G. Butler* and *Harold F. Blum*... 877

The effect of fractionated X-ray dosage upon the mouse testis. I. Maximum weight loss following 80 to 240 r given in 2 to 5 fractions during 1 to 4 days. *Henry I. Kohn* and *Robert F. Kallman*... 891

The radiation recovery factor: preservation by the Polge-Smith-Parkes technique. *David W. H. Barnes* and *John F. Loutit*... 901

On wasted radiation and the interpretation of experiments with chronic irradiation. *R. H. Mole*... 907

Some new experiments on protection against whole-body irradiation. *A. Lacassagne J-F. Duplan,* and *N. P. Buu-Hoi*... 915

Results of exposure of newborn strain HR mice to X radiation. *Margaret K. Deringer* and *Egon Lorenz*... 923

Fertility and tumor development in (C57L × A)F_1 hybrid mice receiving X radiation to ovaries only, to whole body, and to whole body with ovaries shielded. *Margaret K. Deringer, Egon Lorenz,* and *Delta E. Uphoff*... 931

Comparison of thyroid lobes, autotransplanted and *in situ*, in the rat: Growth and I^{131} uptake. *Seymour H. Wollman* and *Robert O Scow*... 943

Some biological aspects of the factor in bone marrow responsible for hematopoietic recovery following systemic irradiation. *Mary B. Brown, Barbara B. Hirsch, C. Susan Nagareda, Sarah K. Hochstetler, William G. Faraghan, Paul Toch,* and *Henry S. Kaplan*... 949

The time factor in inhibition of lymphoid-tumor development by injection of marrow cell suspensions into irradiated C57BL mice. *Henry S. Kaplan, Lincoln E. Moses, Mary B. Brown, C. Susan Nagareda,* and *Barbara B. Hirsch*... 975

Nucleic acid content of the thymic cells of normal and irradiated C57BL mice. *Patricia P. Weymouth, Norman E. Delfel, Ruth J. Doell, Helene L. Steinbock,* and *Henry S. Kaplan*... 981

Electron microscopy of the virus of rabbit fibroma. *Bolivar J. Lloyd, Jr.* and *H. Kahler*... 991

The in vitro initiation of pulmonary adenomas in fetal mouse lung by a single exposure to ultraviolet irradiation of wave-length 2537 Å. *Stanfield Rogers*... 1001

Spontaneous and radiation-induced pituitary adenomas of mice. *Arthur C. Upton* and *Jacob Furth*... 1005

Successful skin homografts after the administration of high dosage X radiation and homologous bone marrow. *Joan Michel Main* and *Richmond T. Prehn*... 1023

Effect of local roentgen irradiation on the formation of new capillaries after injury. *Ruth M. Merwin* and *Elizabeth L. Hill*... 1031

Study of recovery at low temperature of X-irradiated *E. coli* cells. *A. W. Pratt, W. S. Moos,* and *M. Eden*... 1039

Long-term effects of acute and chronic irradiation in mice. I. Survival and tumor incidence following chronic irradiation of 0.11 r per day. *Egon Lorenz, Joanne Weikel Hollcroft, Eliza Miller, Charles C. Congdon,* and *Robert Schweisthal*... 1049

Long-term survival following X-irradiation and the irradiation of the α particles from radon and its decay products. *Joanne Weikel Hollcroft, Egon Lorenz, Marion Matthews,* and *Charles C. Congdon*... 1059

Experiments on the disposition of acutely inhaled $C^{14}O_2$. *Austin M. Brues* and *Agnes N. Stroud*... 1071

Effects of combined local treatment with radioactive and chemical carcinogens. *Arthur M. Cloudman, Katherine A. Hamilton, Ralph S. Clayton,* and *Austin M. Brues*... 1077

Aminoaciduria following total-body irradiation in the human. *Elaine J. Katz* and *Robert J. Hasterlik*... 1085

Protection of mice against X irradiation by plasma proteins. *Agnes N. Stroud, Austin M. Brues,* and *Mildred M. Summers*... 1109

A comparative analysis of radiation lethality in mammals exposed at constant average intensity for the duration of life. *George A. Sacher*... 1125

Level of protein intake and nitrogen excretion in rats following total-body X irradiation. *Julius White, Bernard E. Burr, Harriett T. Cool, Philip W. David,* and *Mona S. Ally*... 1145

Cirrhosis of the liver in rats following total-body X irradiation. *Julius White, Charles C. Congdon, Philip W. David,* and *Mona S. Ally*... 1155